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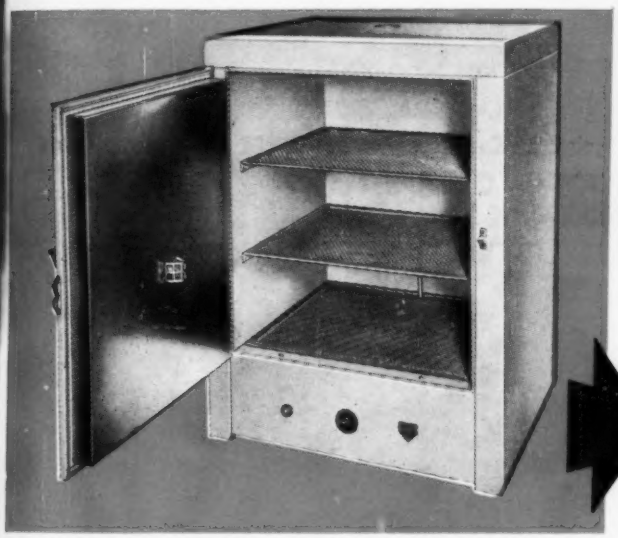

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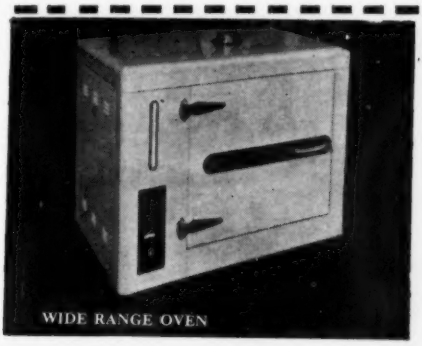

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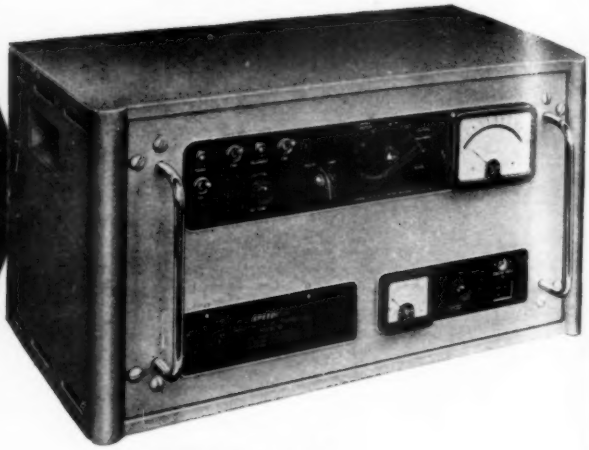

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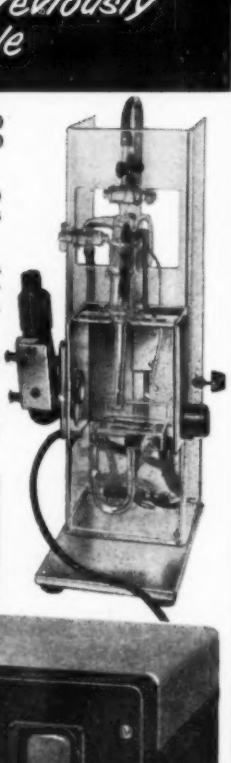

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A COMMONWEALTH ADVISORY AND TECHNICAL SERVICE

THE fourth report of the Select Committee on Estimates for the session 1959–60, which deals with the Colonial Office, is of considerable interest to many scientists and technologists for its section dealing with the Overseas Civil Service*. Of equal scientific interest, however, is the section which examines generally the provision of aid and advice on administrative and technical problems to dependent territories and independent countries in the Commonwealth, and which leads the Select Committee to recommend that a committee of inquiry be set up to consider the establishment of a Commonwealth Advisory and Technical Service. A sub-committee of the Select Committee regarded this as an indispensable instrument of future assistance to such countries inside and outside the Commonwealth. Besides this, the Committee's examination of financial relations with the dependent territories touches on the mechanism by which grants are made for research and development purposes, and finally the Committee's consideration of proposals for some form of merger of the Colonial Office and of the Commonwealth Relations Office are of wide general interest as well as closely affecting the future of the Overseas Civil Service.

Consideration of the arguments for and against such a merger led the Committee to accept the arguments in favour. The Committee believes that it is desirable from the point of view of efficiency in Whitehall, and that it offers the best way of preserving, for the benefit of independent and dependent territories alike, knowledge and experience that might otherwise be lost. The continued existence of two separate Departments of State to deal with the affairs of a rapidly developing and changing Commonwealth leads to a dichotomy of thought and approach that militates against the unity of the Commonwealth; and the Committee does not regard as well founded the argument that such a merger would not be acceptable to the rest of the Commonwealth. On the contrary, it believes that with the right presentation and given sufficient thought and care, the concurrence of the other independent members of the Commonwealth can be secured, and that the benefits to be gained are so substantial that no effort should be spared to achieve it. In particular, the merger would ease many economic and technical problems during the transition to independence and afterwards.

The Select Committee recommends accordingly that the two Offices should be merged on the lines of a tripartite organization outlined in evidence by Sir Hilton Poynton, the Permanent Under-Secretary of State for the Colonies, in which one of the three sections would be organized functionally with no distinction between independent and dependent countries, particularly in the field of technical aid, social services and the like. If the full advantages are to be obtained, this step should be taken at once; and the Select Committee recommends further that the new Department should be known as the Commonwealth Office and should be administered by a single Secretary of State, assisted by three or more departmental ministers. These views appear to be shared by Mr. Kenneth Younger, director-general of the Royal Institute of International Affairs, who gave evidence before the Committee, and from time to time such a merger has already been suggested in Parliament.

If such a merger of the two Offices takes place, the establishment of a Commonwealth Advisory and Technical Service will be no less necessary, in the Committee's view, in order to reach the objectives of planning overseas assistance. Moreover, Sir Hilton Poynton also pointed out that economic, educational and research problems do not really differ according to whether a territory is an independent country or whether it is still a dependent territory, and the disadvantages of the present system are likely to become more obvious as less-developed territories such as Tanganyika attain independence. The proposed Service would establish on a rational and more comprehensive basis the present provision for new forms of technical assistance in the shape of the services of experts, and the Select Committee emphasizes that only if aid for new States is granted in an ordered programme can it be properly effective and hence economical to the taxpayers of the United Kingdom.

Establishment of such a Service will enable the maximum benefit to be gained from assistance given to new States during their dependent days, and it would secure for these countries the experience and knowledge of Overseas Civil Service officers who, on retirement from that Service, could continue in the new Service to employ their talents for the benefit of overseas governments rather than take other employment. Finally, this Commonwealth Service should strengthen the links between Commonwealth countries. Recruitment from the Commonwealth, and especially the older countries, and provision by this means of aid by them to young countries, would broaden the basis of the Service and further understanding between Commonwealth countries. These solid advantages are regarded as outweighing the disadvantages of such a scheme, which would not offer a comprehensive solution to the intractable problem of redundancy which already confronts the Overseas Civil Service, though the Committee believes that the difficulty of ensuring graduated promotion for officers in such a Service could be overcome. It emphasizes, however, that it is essential

* Fourth Report from the Select Committee on Estimates, together with part of the Minutes of Evidence taken before Sub-Committee D and an Appendix, Session 1959–60, Colonial Office. Pp. xxxvii+280. (London: H.M. Stationery Office, 1960.) 14s. net.

to safeguard the flexibility and informality of Commonwealth relationships, and it suggests that the administration of the proposed Service in the United Kingdom would require to be entrusted either to a Commonwealth institution, such as the Commonwealth Economic Consultative Council, or to the proposed new Commonwealth Office.

In considering the financial aspects of relations with the dependent territories, the Select Committee directs attention to the urgent need for devising some system to enable Colonial development and welfare programmes to be brought to completion after the expiry of the period for which expenditure was originally authorized. It also comments on Colonial information services and criticizes the refusal of the Colonial Office to accord information officers in the Colonial territories right of direct correspondence on technical matters with the Central Office of Information. Such rights, it recommends, should be granted, especially in territories approaching independence; and it also recommends that, especially in emergent territories, the British Council should seek to enlist local financial support, as such co-operative effort would enhance local appreciation of the services of the Council.

It is against this background, and especially the two major recommendations for the establishment of a Commonwealth Office and a Commonwealth Advisory and Technical Service, that the Committee's discussion of the problems presented by the Colonial Office staff and the Overseas Civil Service is best considered. Despite the present uncertainty of careers in the Colonial Service, the number of candidates expressing a preference for service in the Colonial Office still exceeds the number accepted, partly because of a decline in the standard of candidates. Nevertheless, the Office at present finds it difficult to guarantee careers with proper expectation of promotion, both to its new recruits and to those more senior officers whose careers can only be continued for a full term of years by transfer to other Departments. While there is less reluctance among its staff than three years ago to spend half their time abroad instead of only the two years early in their service to which officers of the Administrative and Executive Class are normally liable, the Select Committee recommends firmly that only staff prepared to serve at home and overseas should be recruited to the Colonial Office in future. Further, to assist in planning the future establishments, the present staff should be asked immediately to state whether they would be prepared to serve abroad, in the event of their being transferred to the Commonwealth Relations Office. The recommendation that Colonial Office staff should spend longer periods on secondment abroad in order to acquire a fuller understanding of the governments and peoples of Colonial territories is clearly intended to apply to the staff of the proposed Commonwealth Office also.

The examination of the Overseas Civil Services emphasizes further some of the points made by Sir Kenneth Younger in his recent book, "The Public Service in the New States", and is based partly on a memorandum on the Colonial Office and the Overseas Civil Service submitted on behalf of the Colonial Secretary. The numbers of the Overseas Civil Service decreased in 1959 by at least 10,000–14,000, most of whom were employed by the East Africa High Commission and the Governments of Hong Kong, Kenya, Tanganyika and Uganda; but although 1,067 officers were recruited in 1959, at the end of the year there were still 866 vacancies. The main drawback to recruitment is the inability of the Colonial Office to offer a guaranteed career for 25–30 years, owing to the decreasing numbers of posts in territories under the control of the Secretary of State. The suggested Commonwealth Advisory and Technical Service should help to overcome this difficulty, but the Committee is critical of the present conduct of recruitment to the Overseas Civil Service, and it suggests that the Colonial Office should take the initiative among recruiting agencies by revising and modernizing its recruiting methods for such appointments, particularly so as to provide more direct and imaginative forms of contact with university students. It also considers that the Office has not been active enough in seeking recruits from Commonwealth countries, and recommends that the Colonial Office should take immediate steps to promote recruitment from Commonwealth countries to the Overseas Civil Service, both on pensionable and on contract terms. A new approach to the problem of local recruitment is also needed.

The importance of these measures is underlined by events in the Congo and elsewhere during the past year, and it is clear enough that the countries now emerging are likely to have a greater need of the services of overseas officers, because they are less developed than such countries as Ghana and Malaya, the Federation of Nigeria, Cyprus, or the West Indies Federation. Despite the seriousness of the problem of redundancy, which the Committee believes might have been largely solved had the foundation of the Overseas Civil Service in 1954 been more than just a change of name, and would have been alleviated had the Special List *A* and proposed secondment schemes of 1956 succeeded, it is thought that there is a future for the Overseas Civil Service in a much-reduced form, employed in some territories on terms and in an atmosphere difficult to foresee at this time of rapid change. It is essential, in the view of the Committee, for the United Kingdom to shoulder part of the burden of paying differentials between the salary and allowances of an overseas officer and those of a local officer, and this principle has since been accepted.

Such a measure should help to improve the morale and so to staunch the wastage of overseas officers, while retaining the links between the United Kingdom and the Commonwealth and assisting to ensure the stability and forward the development of emergent countries. Of itself, however, it will scarcely do more than alleviate temporarily the uncertainty clouding the future career of every overseas officer in an emergent country, and the Committee recommends further an urgent and comprehensive examination of the organization and terms of service of the Overseas

Civil Service, taken in conjunction with its recommendation for the establishment of a Commonwealth Advisory and Technical Service.

Though the Select Committee presents the case for a single Commonwealth Office cogently and persuasively, if not altogether conclusively, taken by itself it is unlikely to meet the most urgent needs. Far more important than the elimination of overlapping and the financial savings which a new administrative arrangement might permit is the question of outlook and the whole tempo of administration. This is indeed recognized by the Committee, which noted, moreover, that some increase in staff in consequence of the greater need for technical services in the dependent territories might be unavoidable, and again that there was likely to be increasing need for officers with technical rather than purely administrative qualifications. The evidence submitted points clearly to the danger that, without some merger, the existing Colonial Office advisory staff of 58 dealing with such questions as agriculture, animal health, education, fisheries, flora and fauna, medical research, etc., may be dispersed, and also that the important research programmes now maintained from Colonial Development and Welfare Funds may be interrupted when territories come to independence.

The essential recommendations of the Committee are those relating to the Overseas Civil Service and to the establishment of a Commonwealth Advisory and Technical Service, and they have the prior claim on the Government's attention. They are more important than financing the projected scheme of technical aid to which Mr. Iain Macleod referred on October 12 during the Conservative Party meeting at Scarborough, and which is unlikely to be effective unless prior attention is given to the means by which such aid is to be channelled and the conditions of service to be offered to those who are expected to provide such assistance. Unless those conditions are reasonable and appropriate, it may prove impossible to recruit staff of the high quality required, still less to expect from them the standards in which enthusiasm and efficiency are fostered. Like Mr. Kenneth Younger in "The Public Service in New States", the Select Committee puts the emphasis in the right place—on the human factor, on the men and women who will provide the required services, and on the conditions under which they will be expected to work. With this report before it, the Government cannot just leave the problem to such bodies as the newly formed Overseas Development Institute; and in view of the demand that technical assistance makes on the services of scientists and technologists of all kinds, it is to be hoped that the Overseas Research Council, with the full support of the Minister for Science and the Advisory Council for Scientific Policy, will lend the full weight of its influence in the establishment of satisfactory conditions for overseas service, and the urgent and thorough examination of the desirability of establishing a Commonwealth Advisory and Technical Service as the Select Committee recommends.

A CENTURY OF PROGRESS IN EVOLUTIONARY BIOLOGY

Darwin's Biological Work

Some Aspects Reconsidered. By P. R. Bell, J. Challoner, J. B. S. Haldane, P. Marler, H. L. K. Whitehouse and J. S. Wilkie. Edited by P. R. Bell. Pp. xiii+343+5 plates. (Cambridge: At the University Press, 1959.) 40*s*. net.

THIS valuable book consists of six excellent chapters on the present position of a number of the biological topics on which Darwin himself worked. Unfortunately the inept title, which suggests a work of purely historical interest, is likely greatly to hinder sales. This is a pity because it deserves to be in every scientific library and widely read among senior students.

The opening chapter by P. R. Bell, who edits the volume, summarizes a century of research on the movement of plants in response to light. Among the many important conclusions emphasized is the fact that the study of phototropism has become intimately connected with the basic problems of cell physiology —a conclusion which would certainly have astonished Darwin, who at least in his theorizing on natural selection was inclined to regard the animal or plant as 'given', and who was not much attracted to physiology. Bell points out that among the implications of modern views as to phototropism is the conclusion that the current division of botany into two more or less independent disciplines—morphology and physiology—is neither sound nor likely to be profitable. The other botanical chapter also takes the form of a most able review, by H. L. K. Whitehouse, of a hundred years progress in the study of cross- and self-fertilization of plants. One of the most topical subjects which emerges from this discussion is that of cytoplasmic inheritance and the respective parts played by nucleus and cytoplasm in hereditary transmission.

Perhaps the two zoological chapters constitute the most outstanding feature of the book. The first is by J. B. S. Haldane and is entitled "Natural Selection". In a little less than fifty pages Haldane contributes a review of the subject which is masterly in its simplicity and lucidity and yet at the same time profound. It is hard to imagine how it could have been better done. However, the last two-and-a-half pages, which deal with philosophical difficulties and implications of Darwinian theory, are open to some criticism. Here the writing is too condensed, and statements are made without sufficient explanation and support; although it is probable that if he had given himself more space the writer might well have been able to provide these. Thus he states that the difficulty in assuming mind to be a product of evolution does not arise for a consistent idealist, nor for a consistent materialist. Whatever may be the idealist position, this is certainly not true for what is normally understood by the term 'materialist'. Although Haldane has a few words to say as to what he himself means by this term in relation to some Chinese and Hindu philosophical concepts, he does not say enough to make the matter clear. Another idea which it would have been good to have further developed arises from the suggestion that Darwin may have started a revolution in philosophy as great as that initiated by Aristotle, whose logic is mainly based on his efforts to classify animals. Darwin, in comparing species,

pointed out that in studying any two forms we shall be led to weigh more carefully and to value higher the actual amount of difference between them. Haldane suggests that some future philosophy will be based on differences whereas to-day's common-sense philosophy, Aristotelian in origin, is based on similarities. He considers that this may in due course have a profound effect on the outlook both of scientists and philosophers on the natural world.

Among all Darwin's writing "The Expression of the Emotions in Man and Animal" (1872) was perhaps exceeded in originality only by "The Origin" and "The Descent of Man". Its impact was long delayed, but none the less great when it came. P. Marler provides an admirable chapter on subsequent developments in the study of animal communication. He deals with chemical, visual and auditory communication in both invertebrates and vertebrates, and summarizes for the first time in this connexion a great deal of the modern ethological literature. His chapter is profusely illustrated, and the sections on the evolution of bird song, on the origins of communication and on animal and human language are of particular interest. The book is completed by a valuable account of palæontology and evolution by J. Challoner, who pays particular attention to the imperfections of the geological record, the question of group evolution and evolutionary series.

Finally, a chapter by J. S. Wilkie on the originality of Darwin's theory of evolution shows him to be an expert on the work of Buffon and Lamarck, and consequently he is able—in contrast to many modern writers on this subject who take their views second-hand—to give an authoritative assessment of the scattered suggestions as to the possibility of evolution in the works of the former. In regard to Lamarck, he points out that it was his deistic assumptions which gave his theory its peculiar structure. Lamarck supposed that God, in directing the process of evolution, was aiming directly at the production of man and that for this reason linearity should be empirically discoverable in the evolutionary series. It is characteristic of deism to assert that the mind of God is infinite yet to suppose that we can see things from God's point of view. Believing this, Lamarck was unable to tolerate the idea that any species should be 'wasted' on the way towards the production of man so he is unwilling to admit the possibility of extinction. It was these presuppositions and their consequences which made the Lamarckian theory unable to adapt itself readily to the data of comparative anatomy, and even less able to assimilate the fossil record, which of course shows no simple upward trend of organization. Wilkie's chapter brings to a conclusion a valuable and distinguished book.

W. H. Thorpe

RADAR IN WEATHER RESEARCH

Radar Meteorology

By Louis J. Battan. Pp. xi+161. (Chicago: University of Chicago Press; London: Cambridge University Press, 1959.) 45*s*. net.

WHEN it was discovered that centimetric radar could detect precipitation, the meteorologist found himself in the possession of an almost embarrassingly powerful tool for weather research. Since the end of the War, meteorologists in many countries have used the spare military radars which became available to make great advances in precipitation physics. There is a use, too, for radar in weather forecasting, and now that State meteorological services are acquiring their own weather-radar sets, increasing numbers of meteorologists are being introduced to this technique.

There have been few general surveys of progress in this field, or accounts of radar theory from a meteorological aspect, and the newcomer to the subject has been faced with the task of acquiring the basic physical principles from text-books, of finding out the workings of his radar equipment from the technicians and of abstracting past research work from a mass of published papers. This slim volume by one of the foremost radar meteorologists in the United States is written for these, and for others with a more superficial interest in radar meteorology. Although more than one-third of the book is devoted to radar theory, they will learn little physics and less electronics from it. After an all too brief chapter on the fundamentals of radar and the types of indicator units normally encountered, and a sketchy introduction to the theory of propagation of electromagnetic waves, the standard equations for the detection of spherical and non-spherical water and ice particles are produced. While the Rayleigh approximation can be used at centimetric wave-lengths for rain, hailstones exist in sizes well outside the Rayleigh region. It is surprising therefore that no reference is made to Ryde's war-time work showing the very high back-scattering cross-sections of larger ice spheres (recently confirmed by the author's own calculations).

The difficulties encountered in the quantitative use of radar are perhaps too well brought out in the chapter on precipitation measurements. The power received back by the radar is proportional to the sum of the sixth powers of the drop diameters. In order to deduce rates of rainfall or other meteorological data, it is necessary to make assumptions about the drop-size distribution law, and it is encouraging that reasonable results can be obtained when this is done. No attempt is made to explain the large discrepancy between observed and calculated values of echo power which has been found by all those attempting to obtain quantitative values from signal intensity measurements.

The greater and more useful part of the book is devoted to a discussion of the use made by meteorologists of centimetric, and more recently of millimetric, radars. Here the author, being a meteorologist, is on happier ground, and has produced a valuable and concise account of research in this field during the past 10–15 years. Inevitably there is emphasis on American investigations into severe weather, both from thunderstorms and hurricanes, which in themselves are of little interest to some readers. Nevertheless every country has its own weather problems, and their analysis by radar techniques will lead, as this book indicates, to a better understanding of cloud and precipitation physics.

There are two notable omissions in the list of chapter headings on the uses of radar. No specific mention is made of the value of radar to the synoptic meteorologist. While it may be true that the forecaster has no use for radar information, an appraisal of the value of the data for forecasting research would have been of great interest. Neither is there mention of the allied problem of the analysis and dissemination of radar data, a problem which will increase in magni-

tude as radar sets are made to produce more and more data required for use operationally.

A chapter on 'angels' shows that argument is still strong on the causes of echoes received from apparently clear air. The book concludes with a chapter on special instrumental techniques, which might well have come at the end of the theoretical section, since some of the techniques are referred to thereafter. There is no doubt that the meteorologist is now developing radar sets to meet his special needs, and we can expect progress to become even more rapid than over the initial fifteen years of radar meteorology covered by this book.

The book is well produced and there are adequate references, although the method of giving references as footnotes, liberally sprinkled with *op. cit.* and *ibid.*, is at times irritating. The small circle of prospective buyers presumably necessitates the high price.

J. R. PROBERT-JONES

DELINQUENT BOYS AND THEIR PARENTS

On the Threshold of Delinquency

By John Barron Mays. (Social Research Series.) Pp. x+243. (Liverpool: Liverpool University Press, 1959.) 25*s.* net.

Delinquency and Parental Pathology

A Study in Forensic and Clinical Psychology. By Dr. Robert G. Andry. Pp. xv+173. (London: Methuen and Co., Ltd., 1960.) 21*s.* net.

EACH of these books is a study of a small group of delinquent boys—but they have little else in common. Mr. Mays has written an engaging account of an attempt to deal with a particularly difficult bunch of youngsters by setting up a youth club in a delinquent area. No systematic description is given of what actually went on inside the Dolphin Club and one has only a vague picture, for example, of the number of staff engaged, of the use and misuse of equipment, and what a normal week's programme looked like. But a much more important point is made and illustrated with a wealth of detail, that the Club succeeded in keeping boys off the streets and enlisted the co-operation of otherwise indifferent parents in achieving this and possibly other aims.

There are three main strands running through the work: (*a*) an account of incidents and activities in and around the club; (*b*) pen pictures and case-histories of the club members; (*c*) many observations on the social patterns of the district and on the nature of delinquency in general.

The book is somewhat discursive, and the author moves rather too freely from one topic to another. But the observation is shrewd, the descriptions are vivid, and the commentary is uncommonly penetrating. The reader's interest is held, and at the end one has a clear picture of a courageous experiment carried out by people who had no illusions but who were not thereby discouraged. Their achievement is clear and the book is both informative and encouraging.

In a particularly discerning passage (pp. 26–28), Mr. Mays gives an overall estimate of the major factors underlying delinquent behaviour in adolescents. He brings out clearly the difficulties of "boys approaching adolescence who require a strong father figure with whom they can identify themselves". This is, to a large extent, the text of Dr. Andry's remarkable study. Here eighty delinquent boys in a remand home are compared with eighty non-delinquent boys from a reasonably matched group. Following a systematic interview of each of these boys and of thirty sets of parents in each group, he examines parental roles as perceived by the boys and also the parents' perception of their own roles. He concludes that delinquent boys "tend to perceive greater defects in their fathers' roles than in their mothers' roles, whereas non-delinquents tend to perceive the roles of both parents as being adequate. (Further, this seems confirmed in the main by both parents)". This general conclusion is followed up by detailed investigation on specific points, and the differential response of the delinquent and non-delinquent is subjected throughout to a statistical test of significance. For the most part the differences between the responses of the delinquent and the non-delinquent group are most impressive.

In his introduction to Dr. Andry's book, Dr. Mannheim implies that some special interest in fathers is a not unexpected reaction to Bowlby's now classic theory of the effects of early maternal deprivation. This may be so, but Dr. Andry's study and Dr. Bowlby's work are not strictly comparable. Andry specifically excludes neurotic cases (though he does not define the term) and his technique is confined to questioning adolescent boys (between the ages of 11 and 15 years) and some of their parents. Since he excludes (for perfectly good reasons) children with an *I.Q.* below 80 and also children from broken homes, his sample is by no means representative of the delinquent population. He might, quite probably, obtain similar results from a wider sample. Bowlby claims to have shown a clear connexion between early traumatic maternal deprivation and subsequent maladjustment (not necessarily delinquent). Moreover, a high proportion of the cases in which he is interested could properly be called neurotic or psychopathic. This is not inconsistent with, but is quite different from, assessing the respective roles of father and mother during the adolescent period.

Dr. Andry's book is somewhat repetitive and his language is occasionally obscure (for example, "a research worker may use a set of data to derive a maximal system of control with respect to the end object via the possible decision"). But the experiment which he describes is rigorously planned and his use of statistical methods is judicious. A short review of the literature and an extremely interesting bibliography are included. This is a study which will certainly have a considerable effect on future thinking and its direct implications so far as case work is concerned are obvious.

One striking fact is mentioned by both Mays and Andry. The delinquent boy is often keenly aware of his father's defects, and particularly of the poor relationship between boy and father. Father and mother are equally aware of the situation; and all the parties concerned would like to see it improved (though their thoughts and feelings may be vague and confused). Experience strongly confirms this, and it seems that actual teaching of the basic techniques of father–son relationships—and particularly the overcoming of embarrassment on both sides—is often surprisingly effective.

JOHN GITTINS

THE NEW RADIO ASTRONOMY CENTRE OF THE UNIVERSITY OF SYDNEY

By Prof. H. MESSEL

THE Chatterton Astronomy Department of the School of Physics, and the School of Electrical Engineering, have established a Radio Astronomy Centre in the University of Sydney.

Radio astronomy is concerned with observations of the heavens at radio wave-lengths rather than with light waves. It is a relatively new and vigorously growing branch of one of the oldest of the sciences, and Australia has taken a leading part in its development. Almost all the Australian work, so far, has been carried out by the radio astronomy group in the Radiophysics Division of the Commonwealth Scientific and Industrial Research Organization.

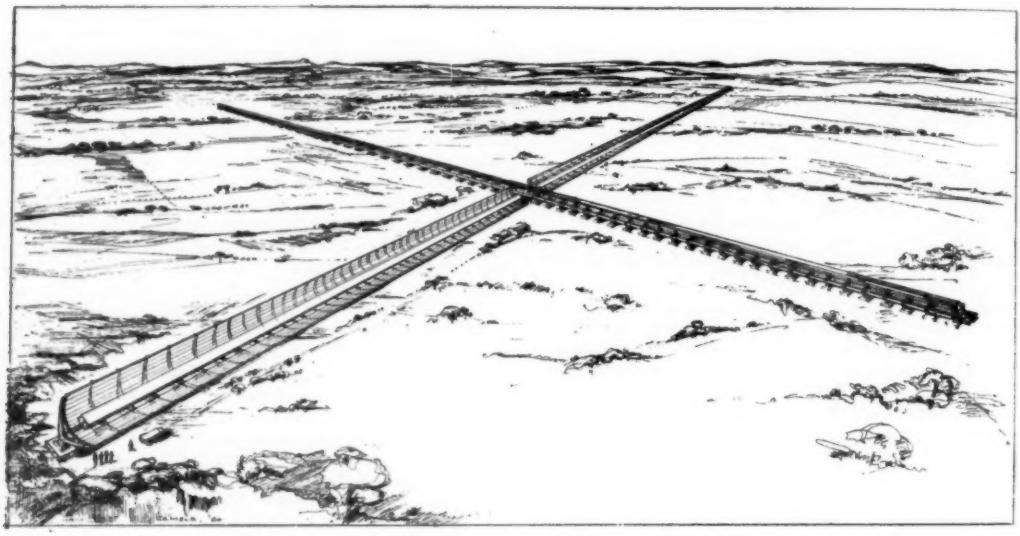

Fig. 1. Artist's sketch of the giant Mills' cross for the University of Sydney Radio Astronomy Centre

Recently, however, several members of this group have joined the staff of the University of Sydney, and, in addition, several outstanding English radio astronomers have already joined, or will soon join, them. Most of these are in the Chatterton Astronomy Department of the School of Physics under Prof. Harry Messel. One group, led by Dr. B. Y. Mills, who was previously with the Commonwealth Scientific and Industrial Research Organization, will engage immediately in radio astronomy work. Another, in which Dr. R. Q. Twiss of the School of Physics and Prof. Hanbury Brown of the University of Manchester will join forces, will work with the giant stellar interferometer in the optical field of astronomy.

Radio astronomy, however, is the meeting point of astronomy, physics and all the most advanced techniques of electronics and electrical engineering. The appointment of a group of leading radio astronomers, including Dr. B. Y. Mills, to the Chatterton Astronomy Department and the recent appointment of Prof. W. N. Christiansen, formerly of the Commonwealth Scientific and Industrial Research Organization radio astronomy group, to the chair of electrical engineering at the University of Sydney, has made possible the establishment of the new Radio Astronomy Centre. The Centre will mean the pooling of resources, intellectual and material, of the Schools of Physics and Electrical Engineering for an attack on some of the major fundamental problems of radio astronomy. The basic interest in the School of Electrical Engineering in solid-state devices and low-noise receivers will contribute a great deal to this joint action.

One of the first problems to be considered by the Radio Astronomy Centre is that on which Dr. Mills has been engaged for some years: the detailed observations of sources of radio emission, both in our own Galaxy and in the very distant regions of space. Such observations are very important for a study of the physics of the rare gaseous medium, lying around and between the stars, which probably holds the key to the problem of the formation and evolution of galaxies such as our own, the Milky Way system. These observations are of particular importance to the School of Physics, which is already established as a leading centre of cosmic-ray research, because it has been shown that there is a very close connexion between cosmic rays and the cosmic radio emissions observed at wave-lengths of 1 m. or longer. It is hoped also that a study of the weakest radio-sources

will yield valuable information about the distribution and state of matter in the most distant regions of the universe and perhaps help to answer the age-old question as to its origin.

For the new studies a very large radio telescope will be needed, far too large to be built in a completely steerable form, such as the large paraboloid being constructed by the Commonwealth Scientific and Industrial Research Organization at Parkes. The radio telescope needed for this work must have dimensions of about one mile, and the most suitable form for this is a cross-like structure invented by Dr. Mills and called a Mills cross. The cost of a radio telescope of this type and size will be about £300,000.

The Chatterton Astronomy Department, supported by the Nuclear Research Foundation, has set aside £100,000 for the first stage of this telescope. The rest of the cost to complete the instrument to its full size must be raised elsewhere by the Schools of Physics and Electrical Engineering of the University. During the next year, when the first stage of the telescope is being designed and construction commenced by Dr. Mills and his collaborators, Prof. Christiansen will be in Holland engaged in a design study for a similar instrument for a Benelux research foundation. Because of the different conditions it is probable that the details of the two instruments will be very different. This close international collaboration is typical of many astronomical projects and should be of great benefit to astronomy in both countries.

Although the design of the Sydney radio telescope has not been completed, the broad specifications are already decided (Fig. 1). It is intended to operate at two different wave-lengths, near 75 cm. and near 3 m. The shorter wave-length is the basic one, and will give the higher sensitivity and resolution; the beam-width will be less than 3 min. of arc with the planned size of 1 mile. The longer wave-length will be used to obtain spectral information, particularly of optical emission nebulæ for which observations in the neighbourhood of this wave-length are most important.

The arms of the cross will be in the form of cylindrical parabolas with 'line' feeds for the two wave-lengths at their foci. It will be possible to direct the instrument in the meridian plane, partly by mechanical and partly by electrical means, rotation of the Earth bringing all parts of the sky, within the coverage of the instrument, into view. Emphasis is being placed on rapid alteration of the setting of the radio telescope so that many selected objects can be studied in a single night's observation; at the same time a number of simultaneous records at closely spaced elevations produces the analogue of a photograph taken with an optical telescope. Because of the vast amount of data which will be produced by the instrument, the principal output will be in digital form on paper tape, and for analysis will be fed directly into *Silliac*, the electronic computer of the School of Physics.

This instrument will combine very well with the 210-ft. steerable radio telescope of the Division of Radiophysics, which is designed primarily for operation at shorter wave-lengths, down to 10 cm., including the *H*-line wave-length of 21 cm. The conjunction of these two very powerful instruments in studies of the southern skies, particularly of the central regions of the Milky Way and the closest of the external galaxies, the Clouds of Magellan, is an exciting prospect for Australian radio astronomers.

The joint effort of the Schools of Physics and Electrical Engineering in this ambitious undertaking is without parallel in the University of Sydney, or indeed in any Australian university, and may help substantially in setting a pattern for the more effective introduction of advanced research into the universities of Australia.

THE INTERNATIONAL GEOPHYSICAL YEAR

A SYMPOSIUM on the "International Geophysical Year", arranged by Section A (Mathematics and Physics) of the British Association, was held in Cardiff on September 1.

Dr. R. Stoneley opened the symposium with a reminder that the idea of international co-operation in scientific matters is by no means a modern one. Sir Graham Sutton has pointed out that in 1861 the American meteorologist Commander F. M. Maury, in an article in the *British Association Notices and Abstracts*, strongly advocated international co-operation in the scientific exploration of the Antarctic. However, the suggestion was not followed up, and it was not until 1882–83 that the First International Polar Year marked the commencement of scientific collaboration in polar research: observations were made in arctic regions of auroræ, geomagnetic and meteorological phenomena. Fifty years later, 1932–33, the Second International Polar Year included as new features the observation of weather conditions above ground-level by means of radio-balloons, and the investigation of the ionosphere by radio.

It was too much to expect scientists to wait until 1982 for another Polar Year, and in 1950, Louis V. Berkner and Sydney Chapman suggested to the Mixed Commission on the Ionosphere that the Third International Polar Year be held only 25 years after the second, with the advantage that 1957–58 would coincide with the expected sunspot maximum. This project was recommended by that Commission to the three parent Unions (Union Géodésique et Géophysique Internationale, International Astronomical Union, Union Radio Scientifique Internationale) and was approved in 1951 by the International Council of Scientific Unions, which appointed a special committee, to be known later as the Comité Spécial de l'Année Géophysique Internationale, with Prof. S. Chapman as president; the name of the project was changed to 'International Geophysical Year', embracing the whole Earth, and not merely the polar regions. The duration of the International Geophysical Year was extended to the eighteen months July 1, 1957–December 31, 1958. By the commencement of the Year the number of nations participating had grown to 67.

The scientific activity was divided among 14 'disciplines': (1) World Days and Communications; (2) Meteorology; (3) Geomagnetism; (4) Aurora and Airglow; (5) Ionosphere; (6) Solar Activity; (7) Cosmic Rays; (8) Longitudes and Latitudes;

(9) Glaciology; (10) Oceanography; (11) Rockets and Satellites; (12) Seismology; (13) Gravity Measurements; (14) Nuclear Radiation. Each of these was represented on the Comité Spécial de l'Année Géophysique Internationale by a reporter. Correspondingly, national committees were set up in the participating countries, with reporters for each discipline. In all, five conferences of the Comité were held, in addition to conferences of special groups. The general plan was that all observational data were to be collected and sent as promptly as possible to the three appropriate World Data Centres, of which the first was in Washington, D.C., the second in Moscow, and the third in centres different for various disciplines. Thus, there should be in existence a triplicate centralized record of the observations relating to each discipline: notification of each despatch of data to the Centres was sent to the co-ordinator of the International Geophysical Year (Vice-Admiral Sir Archibald Day) at Brussels. To co-ordinate more efficiently the taking of observations a calendar of World-Days was drawn up, indicating the days on which there should be a special concentration of observational work. In addition, provision was made for a world-wide distribution of warnings of special events, such as outbreaks of solar activity.

All this central organization, however economically managed, involved considerable expenditure. The earlier expenses were borne by the International Council of Scientific Unions, which by the end of the International Geophysical Year contributed some £14,000 to the project. More than double this amount, about £30,000. was paid by Unesco during the same period, during which the participating nations themselves contributed some £60,000. But this total sum of about £100,000 is small compared with the total expenditure, principally by the participants, of a sum of the estimated order of £300,000,000—a tribute indeed to the efficiency of the central organization.

The task of publishing the results of the International Geophysical Year is an onerous one. A scheme of publication of the *Annals of the International Geophysical Year* was approved by the Comité Spécial de l'Année Géophysique Internationale with the help of an advisory committee presided over by Gen. Laclavère and later by Dr. D. C. Martin, with Sir Harold Spencer Jones as general editor until May 1958, when this task was transferred to the secretariat of the International Geophysical Year. To secure co-ordination of observational work, a series of four "Instruction Manuals" was published well ahead of the Year. The complete series of the *Annals* will deal with the history and planning, as well as national programmes, lists of International Geophysical Year stations, summaries of results with their analysis, interpretation and discussion, bibliographies and catalogues of material available at World Data Centres. Evidently some procedures, such as access to data at these Centres, will need to be developed or modified according to needs and experience. Analogous problems, such as publication and storage of data, will fall to the lot of individual countries. In Great Britain, for example, the Royal Society has well in hand the publication of the Halley Bay Observations, under the editorship of Dr. D. C. Martin.

Something should be said about the continuation of the International Geophysical Year effort after the end of 1958. The manifest success of the plan led to the proposal at the fifth conference of the Comité Spécial de l'Année Géophysique Internationale at Moscow in the late summer of 1958 that the International Geophysical Year period should be extended to the end of 1959, making 2½ years in all. Not all the nations involved could assent to this scheme, since in some cases the national commitment was explicitly limited to the original 18 months. It was, however, unanimously agreed that, so far as practicable, observations should be continued throughout 1959 under the title of "International Geophysical Co-operation", with the central organization bridging the gap until a smaller special committee could take over the functions of the Comité Spécial de l'Année Géophysique Internationale. Some of the International Géophysical Year disciplines that are otherwise catered for have been dropped, and two new disciplines, aeronomy and palæogeophysics, have been included*.

The Antarctic claimed special attention. As H. Wexler pointed out, essentially for seven reasons: (1) It is a large part of our planet; (2) it contains 86 per cent of the world's glacial ice; (3) it is the most efficient cold air factory, and contains the South Pole; (4) the melting ice generates the antarctic bottom current; (5) it is the region of the aurora australis; (6) it contains the south magnetic pole; (7) it presents a stable platform for the investigation of the thermal and electrical properties of an atmosphere cut off from sunlight for many months of the year. Thus, it is not surprising that 12 nations sent expeditions to Antarctica.

During 1954 the Vahsel Bay region of the Weddell Sea was recommended by the Comité Spécial de l'Année Géophysique Internationale at a conference in Rome as a suitable place for a station on the Antarctic continent, and the Royal Society decided to send an expedition there. An advance party late in 1955 erected the base hut at Halley Bay; the main party arrived on January 4, 1957. The leader of the expedition was Col. R. A. Smart, who later was succeeded by Mr. J. MacDowall.

Taking part in the symposium held by the British Association were: Prof. W. J. G. Beynon, who was reporter for the ionosphere, both on the Comité Spécial de l'Année Géophysique Internationale and on the U.K. National Committee for the International Geophysical Year; Mr. J. MacDowall, who, as mentioned, had for part of the time the leadership of the Halley Bay Expedition, in addition to his heavy responsibilities for observations in meteorology, glaciology, seismology and geomagnetism; Dr. G. E. R. Deacon, chairman of the U.K. National Committee for Geodesy and Geophysics, and national correspondent for oceanography.

Ionospheric Observations

Ionospheric studies formed a major part of the overall programme of the International Geophysical Year, and some of the early results of these studies were described by Prof. W. J. G. Beynon of the University College of Wales, Aberystwyth. The most important type of study—vertical radio soundings—was carried out at more than 160 stations. Work on radio-wave absorption by the ionosphere was carried out at some 40 stations by the conventional ground-based method and at a further 30 stations by a method

* The writer wishes to express his indebtedness to Prof. S. Chapman's article, "The International Geophysical Year", published in Vol. 1 of the I.C.S.U. Review, January 1959.

which employs radiation from radio stars. Movements in the ionosphere formed a special investigation at a further 36 stations. This radio work on the ionosphere was supplemented by other investigations, including rocket and satellite experiments.

In siting the ground-based stations, high priority was given to the equatorial zone since earlier work had indicated that the ionosphere exhibits a number of anomalies in the locality of the magnetic equator. These investigations have confirmed that in the uppermost layer of the ionosphere (the *F*2 layer) there is a belt of low electron density centred on the magnetic equator, with maxima located some 15°–20° of latitude away on each side. At times the densities at these maxima are as much as three or four times that in the equatorial trough. Theoretical work suggests that these maxima north and south of the magnetic equator result from a movement of high-level equatorial ionization outwards along the magnetic field, and the results now available from the close network of ionospheric stations tend to confirm this suggestion.

A second equatorial problem, for which the results obtained during the International Geophysical Year have already been published, concerns the 'equatorial electro-jet'—an intense but narrow current system, postulated by magneticians to account for abnormally large magnetic variations observed at equatorial magnetic observatories and notably at Huancayo. A series of rockets launched during the International Geophysical Year by U.S. workers from a ship-borne launcher, at latitudes between 4° N. and 2½° S., show that the equatorial electro-jet is limited to a zone of only about 3–4 degrees of latitude around the magnetic equator, and that it flows in one or more current sheets of thickness about 10 km. at levels of 95–125 km. above the ground.

It has long been appreciated that disturbances in the Earth's magnetic field, and associated disturbances in high latitudes in the form of auroræ, are very probably caused by electrically charged particles entering the Earth's atmosphere from outside and being guided towards high latitudes by the terrestrial magnetic field. More than 50 years ago the Norwegian physicist Störmer made an extensive analysis of the trajectories of solar electrically charged particles as they approach the Earth, and published theoretical curves of particle precipitation in high latitudes. Investigations during the International Geophysical Year, by British workers, of two distinctive polar ionospheric phenomena (the 'radio black-out' and polar sporadic-*E* ionization) have shown that the incidence of these two phenomena and their variations with latitude are of the general form predicted by Störmer's 'precipitation curves'.

The incidence of streams of charged particles at high latitudes might be expected to give rise to an increase in the temperature of the upper atmosphere. Strong evidence that this is in fact the case has come from rocket soundings at Fort Churchill (latitude 59° N.) and at White Sands (latitude 33° N.). Above about 200 km. the average temperature at Churchill is some 1,200 degrees higher than at the lower-latitude station.

Geomagnetic and Seismic Observations

Mr. J. MacDowall gave an account of the Royal Society's expedition to Halley Bay. A suitable site 1¾ miles from the sea on a floating ice-shelf had been found by a ten-man advance party in January 1956, and a living hut 130-ft. long was erected. This was then the largest building in Antarctica, a record which will now undoubtedly be claimed elsewhere. With this advance party in the field during 1956 the main party in Great Britain were able to plan their preparations with a good appreciation of some of the practical problems facing the establishment of the scientific programme. One of the most serious problems was the mutual electrical interference; fortunately this did not greatly affect the carrying out of seismic and geomagnetic observations. The main problem was perhaps the situation of the observatory on what is now called the Brunt Ice-Shelf, which was about 50 km. long and 140 m. thick, floating in about 190 m. of sea water; the relative dimensions can be represented by a board of wood ½ in. thick and 10 ft. long overlying ¼ in. of water.

Such a situation was not considered ideal for seismic and geomagnetic recorders, which are ordinarily built on a firm subsoil-rock if possible. The advance party, the Building Research Station and the Scott Polar Research Institute contributed to the eventual solution of the problem of the geomagnetic instrument foundations, which were formed by driving 13 wooden telegraph-poles 20 ft. into the compacted snow of the ice-shelf; these reduced differential movement and formed a satisfactory vibration-free foundation. Snow accumulated on the flat upper surface of the ice-shelf at the average rate of 3 ft. per year (density 0·36 gm. cm.$^{-3}$); it afterwards became compacted and spread out, with the result that the ice-front, or coast-line, moved outwards, taking the Base with it at about 400 m./year. At the same time the Base rotated by about 0·5° a year, so that regular astronomical observations were needed in order to find the true orientation of the recording magnets.

The seismic recorders were tried in several situations, and were eventually installed at the bottom of a 30-ft. pit, the floor of which was sufficiently firm, and free from local vibration due to the nearby Base: at that depth the compacted snow has the consistency of soft sandstone. In this site the arrivals of longitudinal waves originating in any part of the world were well recorded; secondary phases and shear waves, however, were not well recorded, even from nearby earthquakes in the South Shetland Islands about 1,200 km. away. In seismological work, the first arrivals are undoubtedly the most important and to this extent the seismological readings were quite satisfactory.

On the question of the continuation of observations at Halley Bay, Mr. MacDowall considered that on the observational side the issue is clear cut, as much is now known that was unknown in 1956. In view of the unique situation of the Base it would be highly desirable to bring the Base up to the ideal geomagnetic observatory standard by making quick-run recordings and measurements of space gradients; tests should be made of other and better types of seismic instruments.

Oceanography

Dr. G. E. R. Deacon remarked that the main idea of the International Geophysical Year was to co-operate in work that needs world-wide networks of observations. Oceanographers exploited the idea by studying changes in mean sea-level, long waves

propagated across the oceans, and the general circulation of the oceans.

Mean sea-level, he said, is not the unalterable datum often implied in everyday usage; at the very first meeting of the British Association, indeed, Sir John Lubbock emphasized the importance of studying the effect of barometric changes on the height of the tide. Sir James Clark Ross in the 1840's found that the ocean behaved like a vast water-barometer; variations of temperature and density of course have to be taken into account.

From data compiled by the International Association of Physical Oceanography it was shown by Patullo, Munk, Revelle and Strong that the sea-level is usually lower in spring than in autumn, the range varying from a few cm. at island stations in low latitudes to 165 cm. in the Bay of Bengal. Near the British Isles the mean sea-level is 15–20 cm. higher in autumn than in spring; most of this can be accounted for by differences of temperature and pressure, but part may be due to the storage of water on the land during the winter months. Some of the fluctuations from year to year are attributable to changes in climate, but the actual rise and fall of the land seems to play a part in it. Variations of mean sea-level become of practical importance in coastal defence against inundation.

Another matter of practical importance arises when a seismic surge or tsunami (popularly called a 'tidal wave') travels across an ocean from the seat of a submarine earthquake in the form of waves which cause considerable damage and loss of life when they impinge on the sea shore. Since the wavelength is in practice many times the depth of the ocean, the waves travel with speeds $(gh)^{\frac{1}{2}}$, corresponding to the usual theory of long waves in shallow water, where g is the acceleration due to gravity and h is the depth of the water. Thus, the ordinary speed of travel is 400–550 miles per hour. On April 1, 1946, for example, a submarine earthquake near the Aleutian Is. gave rise to waves which reached Hawaii 4½ hr. later, causing great loss of life and much damage. At Valparaiso these waves were still 5 ft. high, and in the Antarctic were large enough to wash away an old hut left by the British Graham Land Expedition. Since that disaster a warning system, based on the recordings of earthquakes, has been instituted in the Pacific; during the International Geophysical Year long-wave recorders were installed on many oceanic islands as well as on continental margins, and their records have added much to the knowledge and development of the subject.

After 150 years of ocean exploration there are sufficient observations of temperature and salinity at all depths to give a general idea of the large-scale movements of oceanic waters. Dr. Deacon gave as an example the deep current starting between Greenland and Labrador, where cold water with a fairly high content of salt sinks and spreads southwards at depths of 2,000–3,000 metres. The flow is continued, with a reinforcement of water of high salinity from the Mediterranean, as far south as 50° S., where it rises and forms a stratum between a large wedge of Antarctic bottom water and a shallow layer of Antarctic surface water. This current has been traced round the Cape of Good Hope into the Indian Ocean and south of Australia into the Pacific Ocean. These bodies of water, though moving slowly, transport large quantities of heat and considerably affect the mechanics and productivity of the oceans. During the International Geophysical Year a notable advance was made in the direct determination of the velocity of flow by using a simple form of float devised by Dr. J. C. Swallow. This could be arranged to float at a predetermined depth and give out sound signals by means of a simple circuit and a magneto-striction oscillator. The results have been surprising: for example, a southward counter-current below the Gulf Stream was observed to flow at 1/3 knot, water at a depth of 2¼ miles north-east of Bermuda moved northward for several days at nearly 1 knot, while U.S. scientists have found an equatorial subsurface current in the Pacific flowing at 2½ knots.

In all, Dr. Deacon considered the oceanographic work carried out during the International Geophysical Year to be an undoubted success. It brought to light more problems than it solved, and has led to other co-operative schemes.

Conclusions

Summing up the symposium, Dr. Stoneley remarked that while some of the discoveries have been spectacular, such as the van Allen belts, only the lapse of time will permit an adequate appraisal of all the results of the International Geophysical Year. The geophysical conclusions will gradually fall into their place in this rapidly developing science, but, as with the explosion of Krakatoa in 1883, some of the observations will undoubtedly be of value in lines of research as yet quite unforeseen. Many of the benefits are already clear, even if it is premature to assess their relative importance. However, Dr. Stoneley mentioned in particular the following:

(*a*) Support has been forthcoming for large-scale and expensive projects which, without the stimulus of the International Geophysical Year, would not have been undertaken. This is particularly true of Antarctic expeditions.

(*b*) There has been a great stimulus to research in geophysics. In seismology, for example, it has resulted in the setting-up of a number of new stations, of re-equipping and modernizing some of the older ones, and in planning new large-scale lines of research. Likewise, a new magnetic survey of the Earth has been planned.

(*c*) The general public has become much more aware of the background and the development of geophysics. Radio and television broadcasts have been extremely effective.

(*d*) The interrelationship of different disciplines has been emphasized. For example, the determination of the inertial constants of the Earth from observations of artificial satellites, linking up with the gravitational field of the Earth and its internal constitution; the world-wide programme of microseismic observations, linking oceanography, meteorology and seismology.

(*e*) The closer association of scientists, whether of the same or of different nationalities, by correspondence, interchange of publications and personal discussion.

Finally, Dr. Stoneley raised the question of the next International Geophysical Year. His own view was that the rapid advances in geophysics would call for another such project sooner than 1985. However, such occasions of international co-operation while stimulating are expensive. It is also conceivable that an overdose of stimulants may not be in the best interests of geophysics.

R. Stoneley

EFFECT OF NOISE AND VIBRATION ON MAN

THE prefix, bio-, attached to the names of various disciplines among the physical sciences, is becoming increasingly familiar to workers outside the biological field as the specialties in applied biology increase and multiply. One of the sessions in Section G (Engineering) of the recent British Association Cardiff meeting was entitled "Bio-mechanics" and was devoted to two topics in the field of what the Americans are now calling 'bio-acoustics'. Prof. E. J. Richards, of the Department of Aeronautics and Astronautics, University of Southampton, spoke on "Noise Annoyance and its Assessment", and Flight-Lieut. J. C. Guignard, Royal Air Force Institute of Aviation Medicine, delivered a paper on "Some Effects of Low-Frequency Vibration on Man" under the co-authorship of himself and Mr. A. Irving, who holds a research fellowship at the same Institute. Both papers dealt specifically with aircraft noise and vibration.

Prof. Richards began by emphasizing that the primary function of the human ear is communication, and he defined 'noise' as everything which is heard and which hinders communication. He allowed a fairly wide definition of communication and illustrated with several amusing examples how the labelling of a given sound pattern as 'noise' (that is, 'annoying') depends very largely on circumstances and will vary greatly from one individual or community to another. The annoyance caused by jet aircraft noise is, he said, virtually impossible to establish in scientific units and can only be assessed by the reactions of the population in areas irradiated by the noise. He described the establishment of empirical limits of acceptable noise around airports, based on the incidence of complaints by individuals and organized groups living nearby, and cited American surveys of aircraft noise and public reaction to it during operation of piston-engined aircraft from Idlewild Airport, New York (before the introduction of jet traffic). These surveys were used to establish a figure of 107 decibels as the maximum tolerable overall noise-level, measured at ground-level in the open air.

However, Prof. Richards continued, when jet aeroplanes began to fly overhead producing 107 decibels of noise on the ground, it was soon obvious that this figure was no longer acceptable—owing to the different quality of the noise from jet engines. Prof. Richards then digressed to explain the decibel scale of sound pressure measurement—related to the logarithmic response of the human ear to mean square sound pressure—and went on to stress the fact that the disturbance produced by noise depends very much on the frequency distribution of the sound. This is partly due to the non-linear frequency-response of the human ear, and, in practice, it is found that noises of high pitch are considerably more annoying than equally intense noises of relatively low pitch. Prof. Richards showed the meeting curves of equal subjective loudness, together with curves of equal 'noisiness' (annoyance) for pure tones, obtained by American workers in laboratory studies, and he pointed out that the latter curves were, in effect, weighted against the higher frequencies.

The reason for the greater annoyance produced by the jet aircraft (at a given overall sound pressure-level) lies in its emitting a higher proportion of its total noise at high frequencies. For example, jet aircraft noise spectra show the highest levels in the frequency-band 600–2,400 c./sec., as against the band 75–300 c./sec. for the propeller aircraft. Prof. Richards described the concept of the 'perceived noise-level' (quoted in *PN* decibels) and its derivation using the subjective 'equal noisiness' data to transform physical sound pressure measurements. The determination of perceived noise-level allows comparisons between noises of differing spectral quality. A criterion of acceptability can therefore be established for jet noise, based on information regarding other types of noise from industrial sources and piston-engined aircraft. It turns out that the acceptable 107 decibels for propeller aircraft at Idlewild is equivalent to 111 *PN* decibels, which is now the standard accepted by the American authorities and to which jet aircraft must conform in populated areas around that aerodrome.

Prof. Richards then described the probable need for revision and modification of currently accepted *PN*-levels as jet operations became more extensive and the characteristics of jet aircraft and their flight envelopes altered. Present methods of minimizing jet noise include the fitting of noise suppressors and the practice of throttling back the engines when passing over built-up areas during the climb. This of course lengthens the climb and, while reducing the intensity of the local noise nuisance, spreads it over a wider area. Prof. Richards recalled that the current standards of acceptable noise were based on reactions to the worst quartile of propeller aircraft operating over a fairly narrow zone, and that jets would cause a more widespread disturbance. (At the present time, however, the worst annoyance is caused by jet airliners frequently breaking the rules for noise reduction on take-off.)

It is anticipated that the number of jet aeroplane movements at London Airport will increase some eight-fold over the next decade or so, involving not only a very high frequency of take-off during the day but also the introduction of more night operations. Prof. Richards considered some of the implications of these trends for the 111 *PN* decibels-limit. It has been found that, when the incidence of complaints from the surrounding population is related to the number of take-offs at different times of day, proportionally more annoyance is caused by noise during the evening hours of wireless and television entertainment and bed-going. It has been suggested, therefore, that a 10 *PN* decibels reduction in allowable noise must be specified for night operations. It will also be necessary to consider the duration and frequency of occurrence of aircraft noises, and some further reduction in the day-time standard may become obligatory as jet traffic grows heavier.

It appears that quite a high proportion of complaints arise from the landing of jet airliners rather than their taking-off and that in many cases (80 per cent in one survey) people who expressed annoyance revealed, directly or indirectly, some element of fear of aircraft crashes. The pattern of noise on landing is different from that on take-off and is probably more alarming, since the aircraft passes lower over the house-tops, the onset of noise is sharper and the sound spectrum often contains discrete

frequency spikes due to compressor whine. A typical jet aircraft throttled back for landing might show a spike of some 15 *PN* decibels amplitude superimposed on the random noise spectrum at about 2,000 c./sec. It is found that noise containing discrete frequency spikes is more disturbing and would require a lower *PN* decibel figure as the maximum acceptable. The 'annoyance penalty' in the example cited would be of the order of 6 *PN* decibels; but Prof. Richards recommended caution in interpreting such figures, since the estimates of penalty due to discrete frequencies are based on laboratory experiments using 'white' noise, whereas the practical *PN* decibel limit is based on studies of propeller aircraft noise which is itself made up of discrete frequency sounds.

Prof. Richards amplified some of the above points in replying to questions asked in discussion, during which a number of speakers expressed alarm at the prospect of the worsening noise nuisance brought about by jet operations. He agreed that what is urgently needed are extensive and well-controlled social surveys of the noise problem. Speaking on the threat of sonic bangs produced by supersonic transport aircraft, Prof. Richards said that, during a typical supersonic flight, free air pressure jumps of the order of 1 lb./sq. ft. must occur over the majority of the flight path. The nuisance value of such bangs was not at all certain but may be great, affecting large populations if supersonic airliners were to fly overland as well as oversea routes. The problem, again, was one of annoyance and its assessment. Individual reactions to impulsive noise were likely to be very variable. The approach was to determine experimentally the limiting tolerable amplitudes of pressure jump, using realistic samples of people in rather the same way that thresholds of annoyance by continuous noise can be determined in the laboratory and in the field. Prof. Richards felt that, while the aeronautical engineer and the acoustician can do much to minimize aircraft noise at source and in transmission, there is still an urgent need for human data which must be provided by the experimental psychologist and the social scientist.

Aircraft noise disturbs a large population outside the aircraft, whereas mechanical vibration as an environmental factor concerns only the people inside it. Published work on the physiological actions of vibration is accordingly much smaller in quantity, less well known, and of less general interest than that on noise and hearing. The paper by Flight-Lieut. J. C. Guignard and Mr. A. Irving described current research at the Royal Air Force Institute of Aviation Medicine, Farnborough, on the effects of very low-frequency vibrations on man. This is, in the first instance, a problem of military aviation. Aircrew can be violently jostled in high-speed flight through turbulence and, again, disturbing intensities of more or less periodic vibration can occur in some types of aircraft—notably helicopters. In civil aviation, it has been predicted that vibration will be a potential nuisance to the passengers and crew in supersonic airliners. These aircraft may have natural modes of oscillation at very low frequencies, easily excited by gusts and being poorly damped at the great altitudes at which supersonic flight is to be made.

After reviewing briefly the biological actions of mechanical vibration (which are in many cases fairly narrowly frequency-dependent), the authors went on to explain the approach they have adopted in their present field. Although in-flight studies of reactions to turbulence and experiments using dynamic flight simulation have their attractions, the authors favoured the study of human frequency-response to simple sinusoidal vibration. Some reasons were given for this approach, including ease of interpretation and generality of application of the results. The frequency band selected for study was the infra-sonic region (that is, below about 20 c./sec.) in which are excited major aircraft structural modes of vibration, as well as physical resonances in the human body. The lower limit of the band studied was 2 c./sec.

A method of conducting resonance search tests on the human body was described in some detail. One feature which had not been adopted in previous work was careful standardization and control of the subjects' posture (as well as other conditions of vibration) during the tests. Acceleration resonance was measured in seated men at forcing frequencies of approximately 2·4, 4·8 (the dominant response) and 13 c./sec., the latter being detected only as a phase resonance between seat and head. So far, the results showed fair agreement with those of other workers, but the investigation was taken a step further by examining the influence of some physical variables in the conditions of vibration on the resonant response of the body. The influence on resonance of body size, force of vibration (that is, acceleration-amplitude) and muscular tensing were examined and it was found that, while the effects of the first two named were small, tensing modified considerably the man's dynamic response. The tensing manœuvre, in effect, increased both the damping and the stiffness of the body, reducing the dynamic amplification at resonance and increasing the dominant resonant frequency to nearly 6 c./sec.

The paper ended with an account of a simple experiment of more direct practical interest concerning the degradation of visual and visuo-motor performance by whole-body vibration. Performance was measured in four subjects doing two simple tasks during vertical sinusoidal vibration applied through the seat, the test material remaining at rest. The measure of performance used was the time taken to complete a number of replicates of each task. Five frequencies of vibration were applied, in half-octave increments from 2·4 to 9·5 c./sec. at a constant acceleration-amplitude of 0·25*g*. The presentation of conditions was randomized and performance during vibration was compared with that at rest. Vibration hindered the subjects, and the worst performance in each task was measured at 3·4 c./sec. (which is a rather lower frequency than that of the dominant physical resonance in the body). The authors concluded—very tentatively—that the degradation of visual performance in this experiment was related to the velocity-amplitude of vibration at the subject's head. It was incidentally pointed out that breaking down of fixation of a static target by the eyes during whole-body vibration occurs at about 2 c./sec.

Flight-Lieut. Guignard agreed with comments made in discussion to the effect that vertical vibration was only one component of the acceleration pattern of an aircraft in flight, and also stated that different patterns of resonance could be elicited in the body by, for example, transverse excitation. Most work has been done on vertical vibrators as a matter of custom and convenience, and because the vertical components of low-frequency aircraft vibration were considered to be particularly important.

J. C. Guignard

OBITUARIES

Prof. E. M. Killick

Prof. Esther Margaret Killick died on May 31. Her sudden death ended an active and productive professional life, during which she made significant contributions to physiological knowledge, participated widely in academic administration, and was held in high esteem by students and colleagues alike.

Esther Killick came of a medical family, and after her education at Leeds Girls' High School, proceeded to the University of Leeds. Having obtained her medical qualifications, she began her professional career as an investigator to the Safety in Mines Research Board. During this appointment she demonstrated her clinical ability by obtaining the membership of the Royal College of Physicians at the first attempt, without having previously held a house appointment. During her association with the Mines Research Board she came into contact with J. S. Haldane and his work, and her interest in respiratory problems persisted up to the time of her death, when she was still actively engaged in research in respiratory physiology. This interest had a basis in the feeling that her best contribution to medicine would be in the study of the etiology of human respiratory disease, especially in mining and other industries.

Following the work in Leeds, she was appointed lecturer in industrial medicine and hygiene in the University of Birmingham in 1935. Three years later she was appointed lecturer in applied physiology in the London School of Hygiene and Tropical Medicine, and at the same time married Prof. A. St. G. Huggett, professor of physiology at St. Mary's Hospital Medical School. She had two daughters, born in 1940 and 1945. After spending four years at the London School of Hygiene, on the retirement of Prof. Winifred Cullis from the Sophia Jex-Blake chair of physiology in the Royal Free Hospital School of Medicine, she was appointed to this professorship. She thus came to control a department in a school which has a notable record of pioneering educational achievements, and which has, in the past, fostered the cause of education in the sciences for women, when other schools maintained an attitude of indifference, or even of open hostility.

Prof. Killick entered into the problems of her professional appointment with great energy and evolved a well-balanced and highly efficient department, at the same time actively pursuing her own research interests and entering very fully into administrative work. In the course of these activities she contributed, over a long period, research work of high quality, particularly on the effects of carbon monoxide on man and animals. This particular aspect of respiratory physiology and pathology became more and more her special interest in medicine. She was the first to show that the mouse and man acclimatize to carbon monoxide differently, and that the latter does so by the pulmonary epithelium developing the power of actively inhibiting the passage of carbon monoxide from the lungs into the blood, thereby allowing more oxygen to be taken up than would occur if both gases entered by diffusion. This active intervention by the epithelium of the lung explained in an unexpected way the phenomenon which Haldane some years previously had attributed to active secretion of oxygen into the blood. In fact, as Esther Killick showed, Haldane was right in attributing an active role to the pulmonary epithelium, but it was not active transport of oxygen. Her special knowledge in this field was sought widely, both in Great Britain and in the United States, particularly by the U.S. Air Force. She served on various committees concerned with this aspect of physiology, both during and after the War, with the Royal Navy and the Medical Research Council.

In 1953 she worked as a visiting professor in the Department of Medicine at Johns Hopkins Medical School with Dr. R. L. Riley and Dr. Joseph Lilienthal.

She took an active and exceedingly useful part in the administrative work of the University of London. In this she showed exceptional powers of quick and logical thought, and the ability to eliminate the irrelevant and the redundant. She was, in consequence, a most effective chairman, and occupied this position on the Board of Studies in Physiology, and the Board of Pre-Clinical Studies. She was a member of the Board of the Faculty of Medicine, and her advice and opinion were particularly valued on the Senate Committee on Colleges Overseas in Special Relation. Perhaps her work on the latter was her most outstanding contribution to the University, since she participated in the establishment of the Preclinical Departments in the Colleges of Ibadan and the West Indies, and on various occasions served as examiner or visiting representative of the University of London. She also served on the Committee of the Central Research Funds of the University and on the Special Advisory Board on Biophysics.

These various activities illustrate her energetic participation over the whole field of academic work, but they cannot convey the outstanding characteristic of Esther Killick. This was the possession of a quiet manner and a kindly and pleasant disposition which accompanied a keen and quick intelligence and a character of exceptional resolution and determination. These characteristics were discernible in all she did and, in addition, those of her colleagues who have participated in university examinations with her will always remember her quiet approach to the candidates, the kindly appreciation of their problems, and the scrupulous accuracy of assessment of their work.

Esther Killick was guided by certain principles, and one of these was the belief that women, just as well as men, could contribute to society and to a profession by reason of their intrinsic worth; and that they could pursue an active career and simultaneously bring up a family. In both of these she achieved success.

In her work she upheld the traditions of the Royal Free Hospital School of Medicine, the School which appointed in the person of her predecessor the first woman professor of physiology in the University of London. Over the period of nearly twenty years during which Esther Killick worked at the Royal Free, she was held in esteem and affection by her students, her staff and by a wide circle of colleagues.

W. Burns

Mr. L. J. Goldsworthy

Leonard James Goldsworthy was head boy of Ripon Grammar School (1910) and won an Exhibition to Magdalen College, Oxford. Here he became a pupil of T. S. Moore and after graduation (1913) carried out research under the supervision of W. H. Perkin, jun. He took part in College laboratory teaching at Christ Church and Queen's College.

At the early age of twenty-four he became professor of chemistry at the Victoria College of Science, Nagpur, but his scientific work and teaching naturally suffered in the early years of the First World War because of his keen attention to military duties. During 1917–20 he saw active service in Egypt and Palestine as an officer of the Deccan Horse, 16th Cavalry, and held several administrative posts. In later years he continued military activities and during 1933–35 was commanding officer of the 6 (Burma) Batt., U.T.C. Still later he served in the Second World War in the Home Guard in Oxford.

During 1917–22 Goldsworthy held various posts as inspector of schools and deputy or assistant director of public instruction, but in 1923 returned to chemical teaching and research as lecturer at University College, Rangoon; he became officiating professor of chemistry in 1931, retiring in 1935.

While in Burma, Goldsworthy held many official advisory posts and, as was ever his wont, did all that was possible to promote the welfare of his students. He was a warden of one of the university halls of residence and a member of the governing bodies of several university colleges in Burma.

After leaving Rangoon he returned to Oxford and undertook research on intermediates in contemplated synthesis of penicillin and other topics related to natural products, as well as more general fields such as the Leuchs synthesis of polypeptides, orientation of substitutions in benzene derivatives and certain molecular re-arrangements of interest in relation to the chemistry of quinamine. Some of this work was unfinished. In 1937 he found that the Leuchs reaction applied to *d*- or *l*-alanine gave a polypeptide similar to that from glycine, very sparingly soluble and of high molecular weight. But *dl*-alanine under the same conditions gave a water-soluble product of a quite different type. The analyses of ester end-groups indicated a low molecular weight, but it was felt that this required confirmation and other interests supervened.

Later he received a University appointment as senior research assistant to the Waynflete Professor. In this capacity his long experience in the East was turned to very good account, and with characteristic unselfishness and generosity he gave invaluable personal help to many beginners in research and to others who encountered special difficulties. Goldsworthy's own technique at the bench was very neat and his practical example most effective. To overseas students in particular he was not only guide and philosopher, but also friend. Many of them enjoyed his hospitality and that of his wife.

After 1955, one of his major interests was the development of gas-liquid chromatography and, though he retired in 1958, he continued to assist the teaching work at the Dyson Perrins Laboratory and delivered lectures in the academic year 1959–60.

He was a man of great personal charm, a most sincere scientist and a devoted teacher. In 1927, he married Rose Cawthorn and there are two sons.

Robert Robinson

NEWS and VIEWS

Royal Society: Medal Awards

The following awards of medals have been made by the President and the Council of the Royal Society: *Copley Medal* to Sir Harold Jeffreys, formerly Plumian professor of astronomy in the University of Cambridge, for his distinguished work in many branches of geophysics, and also in the theory of probability and astronomy; *Rumford Medal* to Dr. A. G. Gaydon, Warren Research Fellow, for his distinguished work in the field of molecular spectroscopy and particularly its application to the study of flame phenomena; *Davy Medal* to Prof. J. M. Robertson, Gardiner professor of chemistry and administrative head of the Chemical Laboratories in the University of Glasgow, for his distinguished pioneering work on analysis of crystal structure, especially of organic compounds; *Darwin Medal* to Mr. E. J. H. Corner, reader in plant taxonomy in the University of Cambridge, for his distinguished and strikingly original botanical work in tropical forests; *Hughes Medal* to Dr. J. L. Pawsey, assistant chief of the Division of Radiophysics of the Commonwealth Scientific and Industrial Research Organization, Australia, for his distinguished contributions to radio astronomy both in the study of solar and of cosmic radio emission.

National Institute of Sciences of India: Awards

At the annual general meeting of the National Institute of Sciences of India held on October 7, the following awards were announced: *Shanti Swarup Bhatnagar Gold Medal* 1959 (for applied sciences), to Dr. Atma Ram, director of the Central Glass and Ceramic Research Institute, Calcutta, for his outstanding contributions to the development of technology and creating a science consciousness in the glass and ceramics industry in the country; *Chandra Kala Hora Memorial Medal* 1960 (for contributions to the development of fisheries in India), to Sri Hiralal Chaudhuri, research officer (fish breeding), Central Inland Fisheries Research Station, Barrackpore, for his contributions towards induced breeding in carp.

Mining at Leeds: Prof. J. T. Whetton, O.B.E.

Prof. J. T. Whetton, who has recently retired from the chair of mining in the University of Leeds, has had a distinguished academic and military career. After gaining early mining experience in the Yorkshire coalfield he served in the First World War in France and the U.S.S.R.; in 1919 he was awarded the Military Cross and the Russian Order of St. Stanislav. He resumed his mining studies at the University of

Leeds and after graduation was appointed research assistant in the Department of Mining at King's College, University of Durham, where he remained on the academic staff until 1939, becoming lecturer and then reader. During this period he was awarded the degree of M.Sc. by the Universities of Leeds and of Durham. During 1937–48 he commanded the 4th Survey Regiment, R.A.T.A., and was on active service during 1939–45 in the Balkans, the Middle East, North Africa and north-west Europe. He received the Distinguished Service Order, the Order of the British Empire, the Belgian Order of the Crown with Palm and the Belgian Croix de Guerre. Since his appointment in 1945 to the chair, the Department at Leeds has expanded steadily both in undergraduate numbers and research activities. Under his direction work in several fields, but particularly in geophysics, coal preparation, ground movement and pneumatic and hydraulic flow, has been carried out and he has contributed to nearly a hundred papers in these and other mining subjects. He was chairman of convocation for three years and was pro-vice-chancellor of the University of Leeds during 1955–57. He has been chairman of the Committee of Mining Professors of Great Britain for some years, and is at present president of the Midland Institute of Mining Engineers.

Prof. H. J. King

Dr. H. J. King, who is to succeed Prof. Whetton, had several years experience in mining and mine surveying in the South Wales coalfield before 1936 when he was awarded the David Davies Mining Scholarship and other scholarships for studies at the Treforest School of Mines and University College, Cardiff. Before he completed the course he joined H. M. Forces as a Territorial and served with the Royal Artillery and then with the Royal Engineers, specializing in tunnelling, at home and in north-west Europe. For a time before demobilization in 1945 he was staff captain at 21 Army Group with special responsibilities for establishing educational facilities for troops in preparation for their return to civilian life. On demobilization he returned to complete the mining course at Cardiff and in 1946 was awarded the joint diploma of the University College, Cardiff, and the Treforest School of Mines; in the same year he obtained the degree of B.Sc. (Eng.), London, as an external candidate and was awarded first-class honours. He was lecturer in mining in Whitwood Technical College for a year and was then appointed lecturer in mining and surveying at the University of Leeds, becoming senior lecturer in 1956, in which year he obtained the degree of Ph.D. of the University of London. In 1960 he was appointed reader in mining at Leeds. Prof. King is well known for his special interest in mine surveying and particularly for his research work in the laboratory and in the field on ground movement and mining subsidence, in which he has achieved a world-wide reputation.

C.S.I.R.O. Division of Entomology: Mr. F. N. Ratcliffe, O.B.E.

On January 1, 1961, Mr. F. N. Ratcliffe will relinquish his position as officer-in-charge of the Commonwealth Scientific and Industrial Research Organization Wildlife Survey Section to become assistant chief of the Division of Entomology in Canberra. He will be mainly concerned with the ecological and biological control investigations that comprise a substantial part of the Division's activities. Mr. Ratcliffe was a member of the Division of Entomology during 1937–49, working first on termites and later on stored wheat pests. Between 1941 and 1946 he served with the Australian Army Medical Corps, and was actively associated with the work of the Malaria Control Units in northern Australia and New Guinea, and with investigations on mosquito control techniques. On returning to the Division after the War, until the establishment of the Wildlife Survey Section, of which he was the first officer-in-charge, Mr. Ratcliffe acted as assistant to the chief, Dr. A. J. Nicholson, who was succeeded on his retirement this year by Dr. D. F. Waterhouse.

United Kingdom Atomic Energy Authority: Appointments

Senior appointments in the Development and Engineering Group have been announced by the United Kingdom Atomic Energy Authority. Mr. P. T. Fletcher will be deputy managing director (General Management); Mr. R. V. Moore, formerly director of reactor design, will be deputy managing director (Projects); and Dr. H. Kronberger, formerly director of research and development, will be deputy managing director (Development). Mr. S. Fawcett, formerly deputy director, has succeeded Mr. Moore as director of reactor design. The Research and Development Directorate at Risley has been divided into two executive directorates. Dr. H. K. Hardy and Mr. F. W. Fenning have been appointed respectively as director of fuel element development and director of reactor technology.

Solid State Physics at Harwell

The newly formed Solid State Physics Division at the Atomic Energy Research Establishment at Harwell, under Dr. W. M. Lomer (see *Nature*, **188**, 21; 1960), is to be organized into four research groups. One group, under Dr. G. E. Bacon, will be concerned with neutron crystallography, for magnetic and structural investigations and studies in crystal chemistry. Another group, under Dr. R. D. Lowde, will use a variety of techniques, such as time-of-flight measurement and the scattering of cold neutrons, to investigate the dynamics of crystals and of spin systems. Magnetic investigations with polarized neutrons and work on various physical properties employing radioactive tracers, resonance and other techniques will form the work of a third group under Mr. A. D. Le Claire. Later a further group will be added to investigate defects in crystals. There will be opportunity for university participation in the programme.

British Shipbuilding and Research

A statement regarding the replacement of the *Queen Mary* was made on November 3 by the Minister of Transport, Mr. E. Marples, in the House of Commons and by the Paymaster-General, Lord Mills, in the House of Lords. The size, speed, accommodation and physical characteristics of the new ship will be as recommended by the Chandos Committee, and the Government also agrees with the Committee's advice that the capital cost should not exceed £30 million: up to that figure the Government and the Cunard Line will contribute in the proportion of 3 to 2, any excess being met by the Cunard Line. The ship will be owned by a separate subsidiary company of Cunard with provision to ensure that the ship and company

remain under British control. The Chandos Committee proposed that the Government's 25-year loan should carry 4·5 per cent interest, but the Government has decided that interest on the loan should be at the rate for 25-year loans which is being charged by the Public Works Loan Board when the agreement is signed. At the present interest rate of 6·25 per cent, and assuming a total cost of £30 million, the grant would amount to some £3·25 million and the loan to £14·75 million. The statement met with some criticism in both Houses. In the House of Commons, Mr. Marples maintained that the Government was not putting up much risk capital, and that according to estimates of travel for the next ten years, by the end of ten years the taxpayer would be very well covered.

Lord Alexander of Hillsborough again asked for access to the report of the Chandos Committee and Lord Mills referred to an agreement to keep confidential the mass of technical and financial information contained in the report. Lord Lucas of Chilworth, however, while agreeing that there might perhaps be good technical reasons for not producing the report, suggested that the economic survey of the prospects of trans-Atlantic travel which enabled the Chandos Committee to formulate some of its proposals could be made available, and Lord Mills undertook to look into this possibility. The proposal is to be discussed further in both Houses, but dissatisfaction with the Government decision has already been expressed on the grounds that the decision appears to have been undertaken independently of the inquiry by the Council for Scientific and Industrial Research into the shipbuilding industry, and that the report on that inquiry is also being kept secret. Incidentally, when speaking at the launching of the 9,000-ton *Clan Fergusson* on November 3, Mr. A. Cayzer, deputy chairman of the British and Commonwealth Shipping Company, suggested more research by British shipbuilders is necessary, and for that purpose smaller firms might need to amalgamate. A few large substantial companies could carry out research more easily and efficiently than a number of small units, and this applied particularly to the engine-makers.

Children's Interests in Science

To find out children's interests so that the content of a science syllabus could be devised, 141 boys and 142 girls aged eleven in St. Helens were asked to write down a list of questions in science which would interest them and which they would like to know more about. The director of education, N. F. Newbury, has summarized and analysed their replies in the summer edition of *Teaching Science*. Grouped under general headings, the topics dealt with most frequently were natural phenomena (1,186); the human body (753); how things work (718); animals other than humans (393); and in more specialized fields, floating (130); chemistry (130); light (85); inventors (48); magnetism (43). The questions occurring most frequently were of the type "How does an aeroplane fly ?"(100), and "What is electricity and how is it made ?"; while questions such as "Why do people die ?" (27), and "What is gravity ?" (29), were at the other end of the scale. There was only a limited interest on questions on space: 29 questions on rockets, 10 on satellites and flying saucers and 9 on space. Some questions quoted without comment were "How far up does gravity reach ? A candle wants air, so why does it go out when you blow it ? Why does heat make things expand ?" By their replies the children show clearly that, at this stage, they have little enthusiasm for orthodox Nature study, but are mainly concerned with themselves and their surroundings. At this age they tend also to ask similar questions.

Historic Books on Mining and Kindred Subjects

A COLLECTION of rare and historic books owned by Mr. Robert Annan is being exhibited during November 15–December 31 at the Science Museum, South Kensington. These books deal with mining, assaying and metallurgy, mining law and mineralogy. Although mining has a long history, written records are extremely scanty, and it was not until the early and mid-sixteenth century when the works of Agricola and others were published that technical records of mining and ancillary practices became generally available. In medieval times the German miner was pre-eminent, his methods of working the ore deposits and the later treatment of the ore being much superior to those employed elsewhere. It is not surprising, therefore, that many of the rare books in the exhibition are of German origin. Books published in other countries are also to be shown: of particular interest is Gabriel Plattes' "Discovery of Subterraneall Treasure" of 1639, which, although having little technical merit, was the first work devoted entirely to mining to be printed in English. The oldest work on exhibition is "De Mineralibus" by Albertus Magnus (written about 1260) in the version printed at Padua about 1476 and later at Venice in 1495.

The Perkin Centenary Trust: Awards

THE Perkin Centenary Trust was established to commemorate William Henry Perkin's discovery of 'Mauveine' in 1856 and to promote technical education in all aspects of the manufacture and application of colouring matters. The Trustees offer the following awards for the academic year 1961–62: (1) *The Perkin Centenary Fellowship*, for one or two years for the purpose of higher study of any subject approved by the Trustees. Its value is £750 per annum with an additional grant of up to £100 per annum towards certain designated expenses, and is tenable, from October 1961, at any university, technical college or other institution approved by the Trustees. (2) *Two Perkin Centenary Scholarships*, each for two years, renewable at the discretion of the Trustees for one further year, to enable candidates employed in an industrial firm or other institution concerned with the manufacture or the application of colouring matters to receive an education at a university or technical college. Each award will have a value of £350 per annum. There is no means test, and a successful candidate is not debarred from receiving the whole or a part of his normal salary from his employers during his tenure of the scholarship. (3) *Perkin Centenary Trust Travel Grants*. Applications are invited from teachers concerned with the study of any aspect of the manufacture or the application of colouring matters at a university, technical college, or other institution. Preference will be given to applications from lecturers, senior lecturers and readers, or the equivalent grades in other institutions, wishing to gain experience at a similar institution or in industry overseas. Grants will be available towards the cost of travel and maintenance for periods, related to the purpose of

the visit, of from one to three months. Further information can be obtained from the Secretary, The Perkin Centenary Trust, c/o The Chemical Society, Burlington House, London, W.1. Applications for the awards must be made by May 1, 1961.

University News :

Birmingham

THE following have been appointed lecturers: Dr. P. Swinbank, C. P. Van Zyl and Dr. J. D. Dowell (physics); Dr. F. J. Emery (mathematical physics); Dr. T. S. M. Maclean and Dr. E. F. Stack-Forsyth (electrical engineering); K. Foster (mechanical engineering); Dr. G. W. Rowe (industrial metallurgy); Dr. G. M. Bennison (geology); Dr. D. A. Wilkins (botany); Dr. J. M. Webber (chemistry). The appointments to fellowships are as follows: B. W. Petley (physics); H. R. Shaylor (Staff Fellow in nuclear physics); T. W. Preist and T. F. Tuan (mathematical physics); B. Cockayne and Dr. N. Pessall (physical metallurgy); D. J. White (engineering production); D. G. Parsons (S. C. Johnson Research Fellow in the Department of Chemistry). The above appointments took effect on, or before, October 1.

Edinburgh

THE following appointments to lectureships have been announced: B. I. Davies (bacteriology); Dr. P. C. Jocelyn (senior lecturer in biochemistry); Dr. L. G. Plaskett (biochemistry); M. Jacobson (physiology); Dr. A. L. Bartlet (veterinary pharmacology).

Hull

RECENT appointments to lectureships included: Dr. D. W. G. Ballentyne (physics); Dr. J. Friend (botany); S. C. Kapoor (philosophy). Research fellowships have been given to Dr. P. Bruck (chemistry) and Dr. A. Richmond (chemistry).

Leeds

AMONG the recent appointments to lectureships are the following: Mr. P. Kelly (metallurgy), Mr. C. Mack (textile engineering) and Mr. J. R. Proud (agriculture). Dr. D. F. Horler has been appointed as Research Fellow in the Department of Leather Industries and Dr. W. E. Seeds in the Department of Biomolecular Structure. The titles of emeritus professor have been conferred on Prof. P. J. Moir, on his retirement from the chair of surgery, Prof. E. A. Spaul, on his retirement from the chair of zoology, and Prof. J. T. Whetton, on his retirement from the chair of mining.

London

DR. B. J. MASON, Warren Research Fellow of the Royal Society, has been appointed to the University chair of cloud physics tenable at the Imperial College of Science and Technology from October 1, 1961. Dr. S. Cohen, of the National Institute for Medical Research, has been appointed to the University readership in immunology tenable at St. Mary's Hospital Medical School.

Dr. D. R. Laurence, senior lecturer at University College and University College Hospital Medical School, has been appointed to the readership in pharmacology and therapeutics tenable at the College and School; the title of reader in tropical hygiene in the University of London has been conferred on Dr. B. B. Waddy in respect of his post at the London School of Hygiene and Tropical Medicine.

Announcements

THE Keith Prize for the period 1957–59 of the Royal Society of Edinburgh has been awarded to Dr. J. B. Tait, Department of Agriculture and Fisheries for Scotland Marine Laboratory, Aberdeen, for his paper entitled "Recent Investigations in the Faroe–Shetland Channel", published in the *Proceedings* of the Society during the period of the award and for his work in the general field of oceanography.

SIR RICHARD RAMAGE has been appointed Commissioner to examine the present arrangements for localization in the East African Posts and Telecommunications Administration and certain other services of the East Africa High Commission, and to make arrangements for accelerating the process. In particular, he is to consider an extension of the 'in-service training scheme' and qualifications at present required in all grades in which posts are held by expatriate officers.

DR. FRED LESTER, head of the Department of Science at Hatfield Technical College, has been appointed principal of the College of Further Education, Loughborough, and will take up his duties on January 1. Dr. R. F. Robbins, formerly of Nottingham Technical College, and at present senior lecturer in organic chemistry at Hatfield, has been appointed head of the Department of Science as from January 1, 1961.

BESIDES a list of members, with an alphabetical index, and a list of members elected on May 11, the Records of the American Academy of Arts and Sciences, 1959–60, includes lists of officers and committees for 1960–61, the statutes, records of meetings, 1959–60, and the reports presented at the annual meeting on May 11, 1960 (Pp. 128. Boston, Mass.: American Academy of Arts and Sciences, 1960).

THE Institute of Physics and the Physical Society is organizing a conference on "Physics of Polymers" at the University of Bristol during January 10–12. Further information can be obtained from the Secretary, Institute of Physics and Physical Society, 47 Belgrave Square, London, S.W.1.

THE Department of Chemistry, Purdue University, in co-operation with the Office of Naval Research, is organizing a symposium on "Nitro Aliphatic Chemistry", to be held at Lafayette, Indiana, during May 25–26, 1961. Emphasis will be placed on polynitro aliphatic compounds. Those wishing to contribute papers should send abstracts to Prof. Henry Feuer, Department of Chemistry, Purdue University, Lafayette, Indiana, from whom further information can be obtained. Abstracts must be received not later than February 1.

A SYMPOSIUM on "Plant Resources of the Middle East and South Asia for the Pharmaceutical Industry and Rauwolfia", organized jointly by the Government of Pakistan, the Unesco Middle East Science Co-operation Office and the Unesco South Asia Science Co-operation Office, will be held at the North Regional Laboratories of the Pakistan Council of Scientific and Industrial Research, Peshawar (Pakistan), during December 6–14. Further information can be obtained from the Unesco South Asia Science Co-operation Office, 21 Curzon Road, New Delhi, or the Indian National Commission for Unesco, Ministry of Education, Government of India, New Delhi.

THE INSTITUTE OF BIOLOGY

THE tenth anniversary of the foundation of the Institute of Biology was celebrated by a dinner held in the restaurant of the Zoological Society of London on January 8, 1960.

It may be recalled that the first general meeting of the Institute was held at King's College, London, on January 5, 1950, when more than 150 biologists were present, representing most branches of the subject. This meeting, however, was only a culmination of more than three years activity (see *Nature*, **168**, 131; 1951). A council of fourteen members was elected, with Dr. E. Hindle as president, Prof. J. F. Danielli as honorary secretary, and Dr. R. J. C. Harris as honorary treasurer. Rules of membership were drawn up, and an office was established in the British Medical Association House, Tavistock Square, with a general secretary and staff. The membership reached about 500 by the end of the year and has steadily increased so that by the end of 1959 it numbered 2,250, with 260 overseas members.

Each year the Institute has held a symposium on some subject of general biological interest and published the papers and discussions. The first symposium in 1950 was on "Biological Hazards of Atomic Energy", and the topic for 1960 was "Biology of Space Travel". Freezing and drying, biology of deserts, biology of the sea, numbers of man and animals, biological aspects of the transmission of disease, the biology of ageing, the biological productivity of Britain, the effects of pollution on living material, and problems arising from the control of pests and diseases indicate the wide range of topics which have been discussed at successive meetings; and the records of these symposia are of considerable value to students in these respective fields.

The Institute's widening activities necessitated larger premises, and in 1955 a move was made to the present quarters at 41 Queen's Gate, London, S.W.7. At the same time, nine local branches have been founded: six in Britain, one in Northern Ireland, another in Eire, and one in Malaya. These local branches hold meetings independently of the central organization.

The Institute represents the views of biologists on various national and local bodies concerned with technical education, and played an active part in promoting the scheme for endorsed certificates, which is now firmly established with courses in some dozen centres. The Ministry of Education has now joined the Institute in endorsing certificates; and the Scottish Education Department, with the co-operation of the Institute of Biology, has instituted a National Certificate in Biology.

The Institute publishes a quarterly journal containing news of general interest to biologists, as well as articles on broad topics and book reviews. One of its publications, "Biology as a Career", is now in its third edition; in addition, advice on this subject is given to student members, parents, youth advisory officers and teachers. Also two surveys on salaries were undertaken, during 1953 and 1956.

The Institute maintains an appointments register for the use of members seeking advice on potential employers and vacancies, a service of especial value to overseas members. It also publishes a list of full-time consultants on biological subjects, including a list of topics for which the services of occasional consultants can be obtained. The Institute also maintains an information service for the use of members, Government departments, firms and the general public, and is consulted on a wide range of biological subjects.

On various occasions the Council of the Institute has made representations of their views to various Ministers of State on matters affecting the development of biological research. Through the medium of the Parliamentary and Scientific Committee, assistance is given to keep members of Parliament informed of the biological background to such problems as food supplies, ageing, Colonial research, pollution, etc.

The officers of the Institute have been selected with the object of obtaining as wide a representation as possible of the various branches of the subject. The office of president, restricted to two years duration, has been held successively by Dr. E. Hindle, Prof. F. E. Fritsch, Sir James Gray, Prof. W. H. Pearsall and Dr. A. S. Parkes. The honorary secretaries have been Prof. J. F. Danielli, Dr. G. E. Fogg and Mr. H. J. Bunker; and honorary treasurers, Dr. R. J. C. Harris, Dr. N. E. Hickin and Mr. W. V. Harris. The general secretary, Mr. D. J. B. Copp, has served the Institute since 1951, having previously been assistant-secretary of the British Association.

NUCLEAR SCIENCE IN THE MIDDLE EAST

IT might be thought a bold experiment to hold a nuclear science conference in an area which is only just beginning to make progress in this field; nevertheless, the symposium held in Hamadan during July 6–18 was an undoubted success.

The symposium was organized by the CENTO (Central Treaty Organization) Institute of Nuclear Science in Tehran (see *Nature*, **186**, 513; 1960), and all ten of its scientific staff took part. The total number of participants was about sixty, as follows:

France	1
Iran	more than 30*
Pakistan	8*
Turkey	2
United Kingdom	12*
United States	2
United Nations observers	2

* Including CENTO Institute staff.

The French and United Nations participants were experts on various missions in Iran. Delegates' expenses were met from CENTO funds, from the

funds of the Governments concerned, and from other sources.

The symposium was opened by Dr. T. Ziai (Under-Secretary, Ministry of Industries and Mines) on behalf of the Iranian Government, and by the Governor of Hamadan. Thereafter there were nineteen working sessions, including four general discussions, on a wide range of nuclear science topics. The proceedings were informal, and no record was kept; this no doubt contributed to the freedom and liveliness of discussion.

Perhaps the most valuable feature of the symposium was its impact on the nuclear science policies of the CENTO countries. To this end, many of the papers took the form of surveys in which the authors assessed what lines of work might be profitable in their particular field, and what they might involve in facilities and training. For example, Dr. D. I. Page, of the CENTO Institute, considered the application of isotopes to water-flow problems, with special reference to conditions in Turkey. He listed ten problems which had been proposed by the Hydraulics Research Laboratories in Ankara. Of these, four appeared impracticable for such reasons as the large amount of radioactivity involved; two others appeared of doubtful feasibility; and four others, estimation of suspended matter in flood water, detection of seepage points in irrigation channels, movement of underground water, and analysis of cement by radio-activation, were worth pursuing.

Another paper providing useful guidance to countries considering new programmes was that on mutation-breeding in agriculture, by Dr. H. J. Curtis of the Brookhaven National Laboratory. He emphasized the necessity of growing very large numbers of plants in such work; the fact that the variations produced by radiation can also be produced by more conventional means; the need for a lengthy programme of orthodox plant-breeding before a commercially valuable variety can be derived from a radiation-produced mutant; and the meagre results to date in terms of new commercial varieties. Nevertheless, the fundamental aspects of the work fully justify continued effort.

Further papers in the same category were those on prospecting for radioactive minerals, on running a fall-out laboratory, on the use of carbon-14 in biochemistry, and on establishing a medical radio-isotope clinic. There were also general discussions on the potentialities of radioisotope applications in the CENTO region, on their applications in the oil industry, and on atomic energy and public health.

Iran, as the host country, naturally sent the strongest delegation to the symposium, and this gave an opportunity for direct discussion of her nuclear science programme. Prof. A. A. Azad, who is in charge of the Tehran University Nuclear Centre, gave a general account of the programme, which includes a 5-MW. swimming-pool type reactor to be operating in 1962; and Dr. A. A. Sheibani described the Iranian radioactive prospecting project. The discussion which followed ranged over such fundamental issues as the possible uses of a large research reactor, and the value to the region of some of the larger nuclear instruments. The interest aroused was such that both the Pakistani and Turkish delegations expressed a desire for similar international discussions in their own countries.

Other topics at the symposium included reactor instrumentation, waste disposal and recent applications of radioisotopes in medicine, besides various research reports on nuclear physics and on applications of radioisotopes in botany and entomology.

The choice of Hamadan, a quiet, historic town, as the venue of the symposium, rather than Tehran, was something of a novelty to the Iranians, but was much appreciated by all the delegates on account of the relaxed atmosphere and opportunities for informal contacts that it provided. The local authorities contributed to this by their welcome and by their generosity in providing a meeting place, excursions and other amenities. As a final charming gift they gave each delegate a framed picture of a scientist of former days, Avicenna, whose home town was Hamadan.

H. A. C. McKay

CONVECTION IN METEOROLOGY

THE subject of "Convection" was chosen as the topic of the symposium for the first day of the Royal Meteorological Society's summer meeting held in the Electrical Engineering Department, Queen's College, Dundee, on June 30. This was the first meeting of the Society ever to be held in Dundee, and was to be followed on the next day by a symposium on "Agricultural Meteorology" (see *Nature*, 187, 995; 1960). The president of the Society, Dr. J. M. Stagg, took the chair.

The opening speaker was Dr. F. H. Ludlam of the Imperial College of Science and Technology who reviewed the subject of convection in the atmosphere. This is a phenomenon which ranges in scale from the currents associated with fair-weather cumulus to those in the large thunderstorms which result in extensive flooding. Whereas the dynamics of the smaller currents are broadly understood as circulations associated with rising bubbles or columns of warm air, the dynamics of thunderstorms, or for that matter, the precise physical processes involved, are not so well understood. The Bénard convection cell pattern, for which a theory exists, does not seem to occur frequently in the atmosphere, although recent photographic evidence from an American weather research rocket shows a regular pattern of 'groups' of cumulus. The most satisfactory method of forecasting the extent of cumulus development still seems to be that based on the old 'parcel' theory, where the air is assumed to form into bubbles or 'parcels', which break away from the surface and rise adiabatically through the environment until a layer sufficiently stable to prevent further penetration is reached. This method entirely neglects any modification of the environment, such as subsidence warming, and leads to little real understanding of the dynamics of the process.

The difference between a large cumulus extending to, say, 15,000 ft., and a cumulonimbus, with its associated characteristics of hail and lightning, seems

to be more than an enlargement of scale of the same process. A completely new physical factor seems to be involved resulting in up-currents which may be as large as 80 m.p.h. and horizontal currents of up to 60 m.p.h. Radar observations made by workers from the Imperial College of Science and Technology and the Meteorological Office have shown air to move into the base of such a storm at 60 m.p.h., and maintain this speed while passing vertically up through the storm, flowing horizontally out of the top at a height of 7 or 8 miles. The kinetic energy of such a system extending over a horizontal region of about 20 square miles is 100–1,000 times that of a typical large cumulus producing a moderate rain shower. The exceptional rate of growth to a thunderstorm of this size from the typical large cumulus usually occurs after rain has started falling. A physical feature of these thunderstorms which indicates the great energy of the up-draught is the exceptional size of hailstorms produced, which may be as large as golf balls. The latent heat of fusion released during their growth may be an additional source of energy, acting as a kind of booster to convection originated by surface heating.

Many of the vigorous thunderstorms affecting southern England have their origins over northern France and are propagated, rather than simply carried, in the upper-wind system. A strong-wind shear is always associated with these storms and they exhibit a well-defined anvil of cirrus cloud.

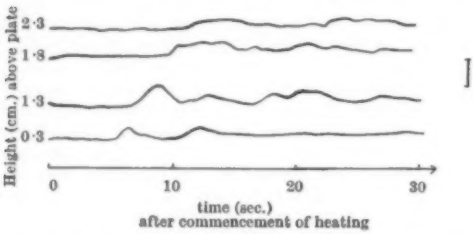

Fig. 1. Temperature fluctuations observed above a heat source in water. Rate of heating of plate = 40 W.

Dr. Ludlam was followed by Mr. C. Dixon of Queen's College, Dundee, who described an experiment he had performed on the initiation of convection currents in water. A semi-circular copper plate 5 cm. in diameter with an embedded electrical heating element was placed on the bottom of a tank of water with the straight edge adjacent to the 'Perspex' front of the tank. Thermocouples were set on to this face at intervals of ½ cm. above the heater and temperature variations at these levels recorded on a Cambridge 6-channel oscillograph. A tracing of a typical recording is shown in Fig. 1. A period of about 10 sec. (depending on the rate of heating) elapsed before any temperature fluctuation was observed, and then an initial pulse travelled past the thermocouples in succession, followed by further pulses at fairly regular intervals of 2–3 sec. On switching the heater off, the pulses died away after a few seconds. It is hoped eventually to photograph tracers to determine the velocity distribution in these currents and associate this with the temperature variations.

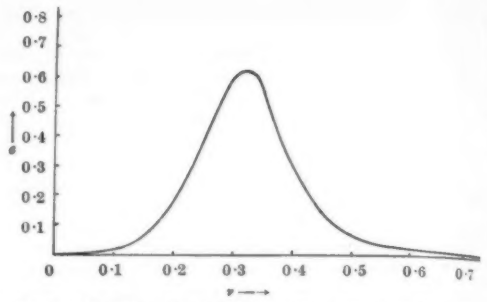

Fig. 2. Absorption of energy by gravity waves at an inversion.
$$e = E/r^4 g\Delta\rho,\ v^2 = \frac{1}{2\pi^2}\cdot\frac{gr\Delta\rho}{w\rho}$$
E = energy absorbed by waves, ρ = density of lower laye, $\Delta\rho$ = density deficit of the upper layer, r = radius of bubble, w = velocity of rise

The third contributor to the symposium was Mr. F. W. G. Warren of the University of Sheffield, who explained a mathematical theory he had developed to determine what proportion of the kinetic energy of ascent of a thermal bubble or column was used in the production of gravity waves at an interface when the bubble or column penetrated a more stable layer. His results showed that such wave production was a maximum when the temperature of the rising column was about midway between those of the upper and lower layers near the interface, that is, such that the bubble was buoyant with respect to the lower layer, but not with respect to the upper. The dependence of the gravity wave-energy on the velocity of the bubble and its size is shown in Fig. 2.

The closing speaker was Dr. R. P. Pearce of Queen's College, Dundee, who explained a theory he had worked out for the development of sea-breezes and which also, with only slight modification, applied to the development of convection currents. The fundamental problem in both these cases is to solve the equations of heating and motion to give the velocity and temperature distributions at any time resulting from a given rate of heat-input. In the case of the sea-breeze this means predicting the time of onset of an onshore wind, and its strength, at any point inland from the coast, given the rate of heating well inland. In the convection problem it means being able to calculate the rates of growth of currents of various sizes from

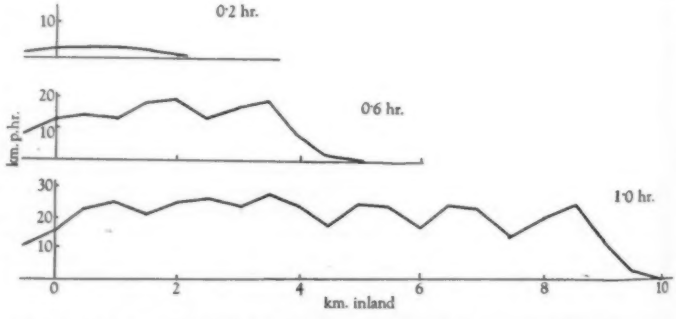

Fig. 3. Sea-breeze development. Surface velocity in km. per hr. Rate of heating 3° C. per hr. beyond 2 km. inland, decreasing towards the coast. Atmosphere initially static and isothermal

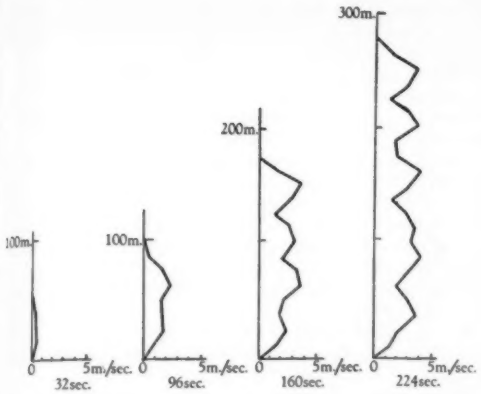

Fig. 4. Development of a thermal. Velocity in m. per sec. Atmosphere assumed initially static with dry adiabatic lapse-rate. Rate of heating approximately 1° C. per 3 min. up to 25 m., 1° C. per 6 min. at 37·5 m. and zero above 50 m.

the known rate of heating that produces them. In both problems the velocity and temperature distributions can be taken as two-dimensional, assuming no variations parallel to a long straight coastline in the case of the sea-breeze, and symmetry about a vertical axis in the case of a convection current. Nevertheless, the mathematical problem is still very complex, involving frequent integrations of Poisson's equation in two dimensions, a time-consuming process even for an electronic computer. A further simplification was therefore made assuming the form of the vertical distributions of velocity and temperature for the sea-breeze, and the radial distributions for the convection current. These assumptions are not unreasonable when the vorticity distribution is elongated, in this case horizontally for the sea-breeze and vertically for the convection current. The result, after introducing boundary conditions and integrating the equations to include the assumed distributions, is to eliminate one of the space variables, leading to a single partial differential equation to be solved for the surface (or axial) velocity u as a function of distance from the coast (or ground) x and the time t. This equation is of the form:

$$\frac{\partial^2 u}{\partial t^2} + a\frac{\partial^2(u^2)}{\partial x \partial t} + b\frac{\partial^2(u^3)}{\partial x^2} = \mathrm{F}(x,t)$$

Here a and b are numerical constants with values depending on the assumed profiles of velocity and temperature. $\mathrm{F}(x,t)$ is the horizontal *gradient* of the heating-rate in the case of the sea-breeze and can be taken as zero everywhere except near the coast. In the case of the convection current, it is the actual heating rate, and this can be taken as zero everywhere except near the ground. The equation is non-linear since no linearizing assumptions are made in the analysis. Some results of the numerical integration, using the Deuce computer at the University of Glasgow, are shown in Figs. 3 and 4.

The initial development of the sea-breeze near the coast and its subsequent penetration inland are features of the result illustrated by Fig. 3, and the analogous generation of the convection current at the surface and its penetration upwards is illustrated in Fig. 4. In both cases the onset at a point away from the origin is fairly sudden and 'frontal' in nature. Another interesting feature is the series of pulses behind the initial one. There is observational evidence for this occurring after the onset of a sea-breeze, and Mr. Dixon's observations described earlier show this property for a convection current in water. These fluctuations may, however, have been introduced into the computation as a result of truncation errors in the finite-difference approximations used in the integration of the equation. Further computations with different heating-rates show that smaller currents develop more rapidly than larger ones, as one observes on a typical convection day when the larger clouds usually develop during the late morning or afternoon.

The symposium ended with a general discussion. Dr. Ludlam's dynamical picture of a thunderstorm was discussed at some length and it became obvious that here was a useful field of study for the dynamical meteorologist, for no mathematical theory which can account for even the broadest features of these storms at present exists.

R. P. Pearce

SAFETY OF FACTORY WORKERS

THE annual report of the Chief Inspector of Factories for 1959 reports a further decline in the number of fatal accidents, from 665 in 1958 to 598 in 1959, the lowest figures recorded this century*. While this figure continues the consistent downward trend in fatalities from the 828 in 1951, the downward trend in total accidents of the previous three years was checked, and the number of reported accidents rose from 167,697 in 1958 to 174,071, in spite of increased activities in the field of accident prevention, although it may be partly explained by the increase in the number of people employed and hours worked. The Chief Inspector, however, finds the increase in the number of accidents to young persons disturbing, and one of two chapters in the report dealing with subjects of interest without being necessarily restricted to the events of the year deals with the prevention of accidents to young persons. This has been discussed in *Nature* of November 5, p. 460; the other special chapter deals with designing machinery for safe operation. Besides directing attention to some of the more important safety principles, which, it is suggested, machine manufacturers should bear in mind, it urges that every opportunity should be taken to bring such considerations before the professional, research and teaching authorities concerned, and that industry as the user should insist on getting premises, plant and equipment with safety 'built in' by the suppliers.

As a result of a considerable number of changes made in the analysis and classification of information

* Ministry of Labour and National Service. Annual Report of the Chief Inspector of Factories for the year 1959. Pp. 128. (Cmnd. 1107.) (London: H.M. Stationery Office.) 7s. net.

about reported accidents, it should be possible to make greater use of the information already provided in the accident reports, and in addition to tables analysing accidents by industry, causation and process, there is included for the first time a table giving accident incidence rates for most of the industries under the manufacturing headings of the Standard Industrial Classification, as revised in 1958. In a number of industries, research associations and other bodies have continued to examine important problems of health and safety, such as dust and fumes and dangerous machinery, and the Chief Inspector believes that other industries could, with advantage, make wider use of their research associations in helping to solve their own special problems in this field. During the year, thirteen inspectors were appointed to the general inspectorate and at the end of the year eight more were awaiting appointment. Twelve inspectors were recruited to the specialist branches, and at the end of the year 409 inspectors of all grades were in posts, but there were twenty-four vacancies in the general inspectorate, one each in the Medical and the Electrical, and seven in the Chemical Branches.

At the end of 1959 there were 222,117 registered factories in Britain, compared with 223,085 in 1958, the decrease being almost entirely in the number of factories without mechanical power. An analysis in March showed that about one quarter of the factory population work in factories employing more than 1,000 people, but only four factories in every thousand are of this size, and on the other hand two-thirds of the number of factories employ fewer than ten people, but less than a thirtieth of the factory population. More than 26 per cent of reported accidents occurred in 'handling goods', 15·1 per cent were caused by falls of persons, and falls of all sorts accounted for 91 fatal accidents in factories, or 26 per cent of all fatalities. A chapter in the report reviewing the year mentions a number of industrial developments which have promoted greater safety in industry.

PHYTOGEOGRAPHY OF THE AUSTRALIAN REGION

DR. NANCY T. BURBIDGE has recently published a comprehensive account and analysis of the principal floristic regions of the vast Australian area, and of selected areas within it of special interest*; she has discussed the composition of the flora in relation to geological and geographical factors, and in a map of considerable interest she has indicated the probable paths of migration, chiefly by way of Sumatra, the Philippines and New Guinea, but including movements within the region, that have contributed to the contemporary phanerogamic vegetation. The phytogeographical region is regarded as including Australia and Tasmania, and can be divided into the tropical zone in the north and east, the temperate zone in the south and east, and the 'Eremæan' zone in the arid centre. While the demarcation of these zones is closely related to contemporary climatic conditions, the respective floras also show the results of selection by former climatic and geographical conditions.

Dr. Burbidge reports that although the flora of the South-West Province shows the highest proportion of endemism, there is a close relationship with the flora of the eastern part of the temperate zone. The Province, in fact, is not "the 'cradle' of the autochthonous elements of the Australian flora though it is apparently an asylum for many relict forms".

At the generic level the Tasmanian flora is not highly endemic, but it shows certain affinities with the flora of Malaysia and with genera found along the Malaysia–New Zealand arc or genera also occurring in South America. Some of the genera are unknown on the mainland, others are known from Australian Tertiary deposits. It appears that the Tasmanian flora includes relics from early Tertiary floras, survivors from the cold-climate regimes of the Pleistocene, and Australian elements which have mingled with the flora during periods of land continuity.

The high rainfall habitats of north-east Queensland are remarkable for the large number of families, genera, and species which are restricted to them, that is, within the Australian region. Thus, many genera have only a single representative which may also occur in Malaysia. Dr. Burbidge considers that some of these may be recent arrivals while others, known as fossils from southern deposits, have a more restricted distribution than in the past. While the close affinity with the flora of New Guinea is noted, Dr. Burbidge points out that there are certain affinities with the floras of New Caledonia and New Zealand which may be independent of those of other parts of the Australian region.

The composition of the Australian flora is primarily attributable to climatic selection within the region and from the biotypes which have become available as a result of migration—migration by communities rather than by chance dispersal of individuals being regarded as a major factor.

The study of Tertiary floras, though not extensive, indicates an affinity with the flora of South America which is "contrasted with the marked lack of data suggesting a similarly increased relationship with southern Africa. The relationship between fossil and modern representatives of such genera as *Podocarpus*, *Dacrydium*, and *Nothofagus* suggests that there has been a northward migration or a withdrawal to warmer latitudes since the Tertiary. All the main elements of the present-day flora are represented in the Tertiary assemblages. If current views on the 'Malaysian' or 'tropical' nature of some affinities and the 'Antarctic' nature of others be accepted then there is no indication that the major migration into the Australian Region came from one particular direction".

* *Australian J. Bot.*, **8**, 75 (1960).

Also comprised in the Australian flora are temperate species with northern hemisphere affinities. Some of these occur in temperate communities in both hemispheres, but others are endemic to, and characteristic of, the 'Eremæan' zone. It is thought that the flora of this zone may have originated from a coastal sand dune and littoral type; and it is suggested that it developed from elements that migrated to the Australian region as early as the Cretaceous when there may have been a coastal continuity with the Tethys Sea. A further suggestion is that these plants remained in coastal habitats but moved inland, possibly along southern estuarine coasts, and became isolated under arid conditions during the Pleistocene or Recent times.

From Dr. Burbidge's analysis, it appears that eastern Australia "has been a very important migration route for a very long period, being linked with the northern hemisphere through Malaysia". The northward migration of Australian species has apparently been less successful than the southward migration of Malaysian ones, the ecological barrier afforded by dense tropical communities having inhibited the northward movement of the light-requiring Australian types: "The significance of such a barrier would be dependent on the climatic conditions during periods of land continnity between Australia and Malaysia and between Australia and New Guinea".

The view that Australia and New Zealand are constituent parts of a biogeographic region is regarded by Dr. Burbidge as unacceptable, unless such a region includes a portion of the island arc in the north-east.

THE WATER NET, *HYDRODICTYON*

THE water net, *Hydrodictyon reticulatum*, occurs only sporadically in Britain, but thanks to its ready growth in culture, the remarkable structure and unusual asexual reproduction of this rare alga have become familiar to many generations of students. Certain details of the life-history were hitherto obscure, however, and even less was known of other species of the genus. Our knowledge of the only three well-defined species, all occurring in South Africa, has now been greatly extended by the recent publication by Dr. M. A. Pocock (*J. South African Bot.*, 27, 167; 1960) of the results of detailed investigations which were in progress for a period of some twenty-five years. In addition to repeated examinations of materials collected in the field, extensive use was made of culture methods, the adoption of agar-plate cultures making it possible to follow the fate of individual cells and cœnocytes.

In all three species of *Hydrodictyon* the basic structure is a network of cœnocytes, each with many nuclei and conspicuous pyrenoids; but whereas in *H. reticulatum* (L.) Lagerheim the mature net is an elongated hollow cylinder, in the other two species (*H. africanum* Yamanouchi and *H. patenaeforme* Pocock) the cœnocytes form a flat typically one-layered plate. In *H. reticulatum* and *H. patenaeforme* the cœnocytes remain cylindrical, while in *H. africanum* they become spherical and dissociate, the individual cœnocytes developing independently for a considerable time.

In addition to these differences in the mature net, the three species differ in the details of their life-histories. It has long been known that in the asexual reproduction of *H. reticulatum* the protoplast of each cœnocyte divides into a large number of uninucleate zooids, which without being liberated rearrange themselves and unite to form a new net in the parent cœnocyte. There have been several differences of opinion as to certain details of this process, but the investigations of Dr. Pocock have clarified many points. In particular, it was found that at one stage in the process the zooids move freely among one another, all protoplasmic connexions between them having disappeared. Although the zooids are confined between the outer protoplasmic membrane and the vacuolar membrane, forming a single peripheral layer, they do not adhere to either membrane. The vacuolar membrane persists throughout net formation, finally disappearing when the young net is liberated by the rupture of the outer wall. There is no asexual reproduction of this type in the other species of *Hydrodictyon*, where daughter nets are never produced within mature cœnocytes.

In *H. patenaeforme* and *H. africanum* the mature cœnocytes produce swarmers which are always liberated into the water. The swarmers of *H. patenaeforme* may either behave as gametes, fusing in pairs to form first a planozygote which finally becomes a hypnospore, or may act as asexual spores producing hypnospores without previous fusion. As in the earlier known sexual process of *H. reticulatum*, the gametes are isogamous. In *H. africanum* the gametes are anisogamous and there is still some doubt as to whether they can act as asexual spores. The asexual production of hypnospores is found only occasionally in *H. reticulatum*.

The hypnospores of all species, whether sexually produced (zygospores) or asexually (azygospores), may enter a prolonged resting phase or may germinate at once following a period of growth. In *H. reticulatum* and *H. patenaeforme* the hypnospores on germination produce comparatively large zoospores, one from azygospores, typically four or eight from zygospores. After a period of motility, each zoospore settles down and gives rise to a polyhedron stage. Following a period of growth the polyhedron in turn divides to form zooids, which unite to produce the germ net. In *H. africanum*, although polyhedra are occasionally formed, the zoospore and polyhedral stages are usually cut out of the life-cycle. In this species the germ net is produced directly on germination of the hypnospore.

From the details of the life-history and from what is known of the cytology of *H. reticulatum*, it seems clear that in all three species the net is haploid, with the zygospore as the only diploid phase in the life-history, reduction division occurring on germination of the zygospore. The polyhedron is an interpolated vegetative phase, peculiar to the family Hydrodictyaceae.

A. ALLSOPP

ROLE OF THE GRAIN COAT IN WHEAT GRAIN DEVELOPMENT

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THE mature grain of wheat has a thin coat consisting of several layers of dead and obliterated cells, and these layers are singularly low in content of various enzymes[1,2]. During the early development of the grain, however, such layers seem to be endowed with specific metabolic functions.

The first indication of this possibility arose from an attempt to correlate the ontogenetic pattern of glutamyl transferase content with the synthesis of gliadin in the developing wheat grain. A correlation was expected on the grounds that gliadin is particularly rich in glutamine[3] and that glutamyl transferase is believed to be identical with glutamine synthetase[4], and therefore responsible for glutamine synthesis. Moreover, it was considered possible that the transferase mediates the incorporation of glutamyl residues into the peptide chain of gliadin, which seems to contain *iso*glutamine[5]. However, when the determinations were carried out in 1958, with the variety Insignia, a rather different picture emerged (Fig. 1). The enzyme showed an early rise of activity, which began to fall 12 days after anthesis, that is, at a time when gliadin had scarcely begun to be produced. The peak of activity was not an artefact due to gradual accumulation of inhibitors or interfering enzymes. This was concluded from assays performed on deep-frozen material in which low activities of late harvests and high activities of early harvests appeared additive when the extracts were brought together.

In the 1959 season, closely similar observations were made on a different wheat variety, Festival, that is, a peak of activity of glutamyl transferase was found at 12 days after anthesis.

At this stage of the work it was realized that the different parts of the developing grain show a complex histogenetic pattern. Nutman[7] had directed attention to some of these phenomena and their possible physiological significance in rye. On macroscopic inspection, the most striking feature is the early growth of the grain coat, followed by its gradual decline and obliteration (Fig. 2); the endosperm shows a continuous growth in weight and volume. The peak in glutamyl transferase activity therefore seems to reflect changes in the coat rather than in the endosperm. This consideration led to investigations of the localization of the enzyme.

The complex composition and ontogeny of the outer layers of the wheat grain have been dealt with by Kudelka[8] and Percival[9]. It comprises the pericarp, integuments and nucellus. Lacking any precise term, the term 'grain coat' has been adopted here for the whole structure. During the earlier stages of development this can be easily separated from the endosperm. Also, from about 8 days after anthesis, the outer pericarp can be separated from the rest of the grain coat.

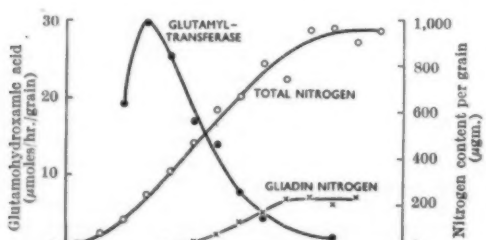

Fig. 1. Wheat, variety Insignia. Time-course of glutamyl transferase activity, and of total- and gliadin-nitrogen content on a grain basis.

From the ears of a particular harvest day the first grain out of a spikelet from the centre of the ear was used. The grains were ground in a cold mortar and three times extracted with water. After centrifugation at 500*g* the accumulated supernatants (1 grain per ml.) were used for the enzyme assay, in which the formation of glutamohydroxamic acid is measured under conditions similar to Stumpf *et al.*[6]; the residue was extracted three times with 70 per cent ethanol, and gliadin was precipitated by adding 5 volumes of a mixture of 2 parts ethanol, 1 part ether and 0·01 per cent lithium chloride

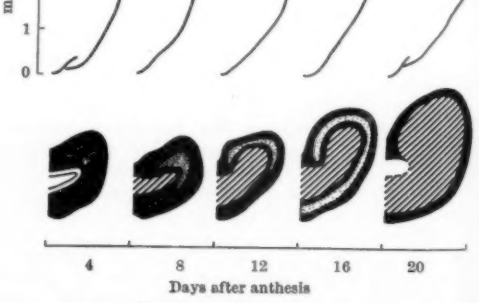

Fig. 2. Wheat, variety Festival. Right halves of abaxial and trans-sectional views of fixed grains harvested at 4-day intervals from anthesis. The blackened parts belong to the 'grain coat'; the gap (stippled) between outer pericarp and 'rest' of the grain coat, present from about 8 days after anthesis, is closed again at day 20. The endosperm (hatched) is at first only an acellular bag (see day 4)

Table 1. Distribution of some Enzymes in the Developing Grain of Wheat

	Glutamyl transferase		Phosphatase		Leucine activating enzyme	
	Δ O.D. 540 mμ	%	Δ O.D. 400 mμ	%	Δ% exchange	%
Outer pericarp	0·303	60	0·149	50	nil	nil
Rest of coat	0·201	40	0·092	31	2·17	29
Endosperm	0·002	nil	0·058	19	5·39	71

Deep-frozen wheat grains, variety Festival, harvested 16 days after anthesis. Observed enzymatic activities of extracts of different parts of the grain, and the corresponding percentages of the total activity of the whole grain.

Glutamyl transferase: change in optical density (Δ *O.D.*) at 540 mμ (ref. 6) after incubation for 20 min. (30° C.) of extract equivalent of 0·5 grain.

Phosphatase: change in optical density at 400 mμ after incubation for 15 min. (30° C.) with disodium *p*-nitrophenol phosphate[10] of extract equivalent of 0·5 grain.

Leucine-activating enzyme: change in per cent exchange between pyrophosphate and adenosine triphosphate[11] after incubation for 15 min. (30° C.) of extract equivalent of 16 grains. Extracts were eluted phosphate gel purifications[12].

This 'rest' notably contains the chlorophyll layer, which is part of the pericarp. The outer pericarp of the earliest stages is opaque white, partly concealing the chlorophyll layer, but becomes more translucent as the grain starts to fill.

Glutamyl transferase activity was determined on extracts of outer pericarps, of 'rests' of grain coats and of isolated endosperms of deep-frozen 1959 material taken 16 days after anthesis. On a grain basis, 60 per cent of the activity appeared to be localized in the outer pericarp, 40 per cent in the rest of the grain coat and none was found in the endosperm. Neither was any activity found in isolated endosperms taken 32 days after anthesis. However, activity was found in concentrated extracts of endosperms, equivalent to 10 grains per ml., taken 12 days after anthesis. This amounted to 2 per cent of the activity of the grain coat. These results were confirmed using fresh material.

These observations suggest that the early peak in glutamyl transferase is associated with the early development of the grain coat and its gradual decline thereafter. They also suggest that the grain coat assumes a predominant role in the synthesis of the glutamine which is synthesized inside the grain. Admittedly this constitutes probably only part, and perhaps even a minor part, of all the glutamine which is ever present in the grain from anthesis to maturity, because the main source is presumably elsewhere in the plant.

The observations of Table 1 provide further examples of the differential distribution of enzymes within the grain and indicate the probable importance of the grain coat in wheat grain development.

The distribution of phosphatase, at 16 days after anthesis, was: outer pericarp 50 per cent, rest of grain coat 31 per cent and endosperm 19 per cent. These figures may be compared with those on phytase of the mature grain[2], where the coat contributes 7 per cent.

Leucine-activating enzyme appeared to reside mainly in the endosperm (71 per cent), none was found in the outer pericarp, but there was considerable activity in extracts of the 'rest' of the grain coat (29 per cent). This contrast between the parts of the coat is probably due to the chlorophyll layer in the 'rest' of the coat. This is full of chloroplasts, and these have been shown to harbour amino-acid activating enzymes[13,14].

These investigations are being continued.

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ANATOMICAL EVIDENCE FOR OLFACTORY FUNCTION IN SOME SPECIES OF BIRDS

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THERE is good morphological evidence that inherent responses to chemical sensory stimuli are of vital importance to some birds. It seems curious that the large olfactory organs of certain species[1] have so often been pointed out by anatomists, yet most olfactory learning studies have been done on feebly equipped birds such as pigeons, and have tended to keep alive in the text-books the idea that the chemical sense in birds is minimal or lacking. The maze type and conditioned reflex studies on microsomatic forms and the succession of uncontrolled field-tests initiated by those of Audubon[2] have kept dust from settling on the question, but have proved nothing about birds with superior receptors. There has been one recent, controlled, statistically significant laboratory experiment on olfactory learning in pigeons[3], and even in these weakly endowed birds the results were positive.

In dissecting the nasal tissues of birds for work on natural defences against respiratory disease[4], I have been repeatedly impressed by the size of some of the olfactory conchæ. Species were selected on the basis of the exposure of their nasal fossæ to extremes of environment, and not on the basis of olfactory behaviour. Of the species thus far dissected, by far the largest and most heavily innervated organs have been seen in the turkey vulture, *Cathartes aura*, the Trinidad oilbird, *Steatornis caripensis*, and the Laysan and black-footed albatrosses, *Diomedea immutabilis* and *D. nigripes*, each of which types represents a separate order with quite dissimilar feeding and nesting habits. Turkey vultures are diurnal carrion

feeders the solitary nests of which are found in sequestered hiding places; oilbirds feed nocturnally on fruits and nest colonially in total darkness in deep high caves in which they navigate by echo-location; the two pelagic albatross species feed mostly on marine crustaceans and nest colonially on islands, in the open, in shallow scrapes.

There is no question of degenerate or indifferent function of the olfactory organs of these birds. Other species seen in the course of this work have 1st nerve equipment varying from modest to considerable, but that of the three specified types is outstanding. In reviewing the literature on the habits of these particular birds, it is apparent that no definitive controlled experiments on the function or the sensitivity of the chemoreceptors have been done. It may be useful, however, to cite a few records of apparent olfactory behaviour in these species as clues to possible functions of the organs to be described.

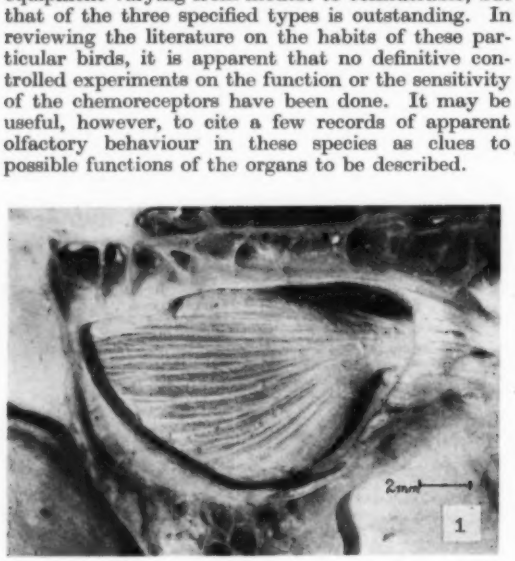

Fig. 1

Turkey vultures are high-altitude hunters. They apparently detect food odours which rise on the thermal updraughts on which they glide. They have been seen to pass over carrion upon which they would have spiralled straight down to feast if vision was the stimulus; having overshot the carrion until the scent was evidently carried up on the wind at an angle, they then turned suddenly and followed the scent beam to the prey[5]. A flock of 40–50 turkey vultures was seen to come in from a communal roost two miles away, sailing into the scent-laden trail of the first morning thermal to feed on offal which had been uncovered at dawn[6]. Fisher[7] has reported that a turkey vulture will not feed on newly discovered carrion so long as a human observer is upwind of it, no matter how well concealed he thinks himself to be.

From the many eye-witness accounts of keen olfactory perception in albatrosses, Loye Miller's is selected because his observation cruise was planned and the birds were marked[8]. Black-footed albatrosses were marked during movements of an oceanographic vessel to six stations 30 miles apart, with a 2-hr. stop at each point. Bacon drippings poured on to the surface of the sea attracted marked birds from at least 20 miles away within an hour. The birds discriminated by olfaction between paint scum and bacon drippings, refusing to approach the paint flux but going directly to the drippings.

The function of the chemoreceptor in *Steatornis* has not yet been determined. Dr. D. W. Snow, who has been studying the oilbirds of Trinidad for several

Fig. 2

years, writes in a personal communication that olfaction may be important in food finding: "Where the olfactory sense may come in, I think, is in locating trees that are in fruit". Some of their food trees are highly aromatic, and ripe and unripe fruits are mixed in the bunch. These nocturnal birds have large blue light-sensitive eyes, and it is not known whether they can distinguish ripe fruits by vision. Oilbirds not only nest colonially but also communally with bats, in absolute darkness, like the oriental swiftlet *Collocallia*. Novick[9] has recently suggested that the swiftlet may need a supplement to echo-location in discriminating its individual nest in a noisy crowded colony and that olfaction may serve this need. Many animals use musk secretions to mark territories, breeding sites, and family members for olfactory identification. Most species of bird secrete musk in their pygial glands, and these glands are known to be hyper-active in some species during nesting (hoopoes, wood-hoopoes, musk ducks, etc.). Turkey vulture nests smell of musk[10], and the musk secreted by albatrosses and other petrels clings to nest sites and even to dried skins for years[11].

The three species illustrated in Figs. 1–3 each has nasal fossæ precisely engineered to filter, warm, moisten, and sample inspired air chemically. Unfortunately, a sagittal view gives no concept of the airflow, nor does it demonstrate that the olfactory chamber is a cul-de-sac enclosed on three sides. The general arrangement of the structures in all the specimens is consistent with those of the oilbird

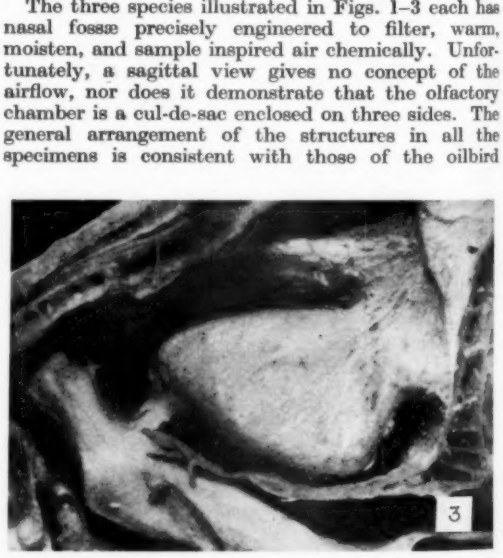

Fig. 3

(Fig. 2), though naturally the conchæ vary somewhat in relative size. The anterior concha, peculiar to birds, serves mostly as a baffle and thermo-regulator; the middle or respiratory one is covered with mucus-producing and ciliated cells; the posterior, olfactory, concha supports the free receptor fibrils of the 1st nerve and a quantity of serous glands peculiar to the olfactory organ. In each species the olfactory concha is conspicuously yellow in the fresh state. This concha is hollow in the albatrosses and the oilbird, the concavity being in communication with the cephalic air-sac system. The vulture concha is scrolled two and a half times, and has therefore a much greater surface area.

The mucosa of the conchæ was gently scraped away to reveal the underlying fibres of the olfactory nerve branches. As shown in Fig. 1, prominent branches are distributed over the entire convexity of the albatross concha. The network of end branches and fibrils was so thick in the turkey vulture that it was not possible to prepare a tidy dissection, and, as Fig. 3 shows, the entire surface was shaggy with torn nerves. The oilbird was photographed before the fibres were dissected out. The branches are somewhat closer together than those of the albatross but not so crowded as those of the vulture. In each species, strong branches of the 1st nerve also lie along the roof, posterior and ventro-lateral walls, and upper portion of the septum nasi of the olfactory chamber.

Though experimental results are wanting, it is suggested therefore that on the combined evidence of morphology and field-observation, it is probable that birds possessing large olfactory organs may respond instinctively to chemical stimuli, either alone or in complex interaction with auditory, visual, vibratory, and other organs of special sense.

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HYPOTHETICAL PATHWAY OF NITROGEN METABOLISM

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THE concept is widely accepted that nitrogen which enters the body of mammals as food ultimately is either stored in the form of body protein or eliminated. Elimination occurs chiefly through urine and fæces, in the form of urea, ammonia, creatine, creatinine, uric acid, amino-acids and other known nitrogen-containing katabolites. This concept represents the rationale for the technique of nitrogen balance studies, in which the nitrogen, recovered from urine, fæces, hair, and cage wash, and measured according to one of the many available modifications of Kjeldahl's method, is subtracted from the intake.

It is implicit in such a concept that positive nitrogen balance values must, within the limitations represented by concomitant variations in the amounts of other body constituents, particularly water and fat, be paralleled by a proportional increase in body-weight. A factor of 30 has repeatedly been found adequate in converting nitrogen weight into 'live' weight of the animal, provided unusually large deposits of adipose tissue are not present[1].

In the present article, results are presented which are not immediately consistent with the accepted postulate. Large and long-term nitrogen 'retentions' have been seen in our animals without proportionate increase in body-weight. Analogous results have already appeared in the literature[2–7] concerning several species, including man. My results, reached independently, confirm earlier observations and add

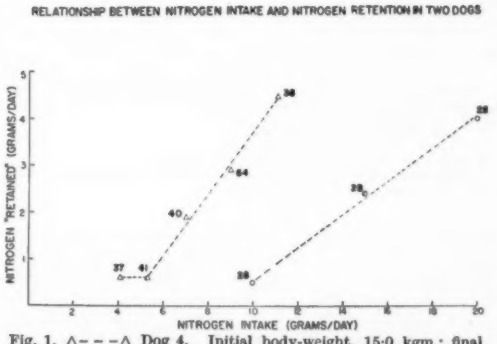


Fig. 1. △– – –△ Dog 4. Initial body-weight, 15·0 kgm.; final body-weight, 15·1 kgm. o– – –o Dog 9. Initial body-weight, 13·0 kgm.; final body-weight, 13·0 kgm. Numbers beside points indicate number of days observed.

Table 1. Nitrogen Balances and Variations in Body-weight in Male Mongrel Dogs

Dog	Status	Days observed	Daily intake: Nitrogen (gm.)	Daily intake: Calories	Total nitrogen 'retained' (gm./dog)	Init. body-weight (kgm.)	Weight gain (kgm.): Expected*	Weight gain (kgm.): Found
9	Normal	100	15·0	1,200	300	13	+ 9·0	0·0
4	Castrated	140	9·0	700	441	15	+13·2	0·0
2	Castr. and Depancreat.	240	20·0	1,727	525	16	+15·7	+0·2
3	Castr. and Depancreat.	240	20·0	1,727	705	15	+21·0	+0·5

* Nitrogen 'retained' × 30.

Table 2. Relationship Between Nitrogen Intake, Nitrogen Balance and Body-weight in Dog No. 4 (Initial body-weight 15 kgm.)

Period	Daily intake Calories	Daily intake Nitrogen (gm.)	Days observed	Average nit. balance (gm./day)	Body-weight (kgm.) Init.	Body-weight (kgm.) Final
1	800	11·10	36	+4·48	15·0	15·0
2	700	8·98	64	+2·88	15·0	15·6
3	540	7·03	40	+2·35	15·6	15·6
4	400	5·30	41	+0·60	15·6	15·2
5	340	4·20	37	+0·62	15·2	14·5
6	396	4·20	22	+0·63	14·5	14·5
7	500	4·20	68	+0·70	14·5	15·1

new elements to this problem of impossibly high cumulative nitrogen 'retentions' without gain in weight. A hypothesis is advanced to explain the anomalous behaviour.

Dogs: Male mongrel dogs were kept in metabolic cages on a supplemented raw horse meat diet, and the nitrogen balance measured as described previously[8]. It was observed that animals, the body-weight of which remained practically constant throughout the experiment, were 'retaining' considerable amounts of nitrogen over long periods of time, as indicated in Table 1. The amount of nitrogen 'stored' by these animals is so large it could not have been retained at the expense of other body constituents. To explore this phenomenon further, the effect of varied nitrogen intakes was studied. Two dogs were observed, and the results are summarized in Table 2 and Fig. 1. Reasonable nitrogen equilibrium was obtained over a limited range of nitrogen intakes (periods 4–7). Above that range, a direct relationship existed between intake and 'retention'. No concomitant increase in body-weight was observed even when more than 4 gm. of nitrogen was 'retained' daily for 36 consecutive days.

Table 3. Composition of the Diets used in the Rat and Mouse Experiments

Diet	*A*	*B*
Composition (per cent)		
Alphacel*	1·8	1·8
Sucrose	20·4	20·4
Corn starch†	40·7	20·4
Casein‡	16·7	37·0
Primex	7·4	7·4
Wessons Salt Mix*	3·7	3·7
Yeast§	9·2	9·2

Vitamins E and D were added to the feedings in the form of 10 per cent tocopherol in cod liver oil (2 drops three times a week). 1 gm. of either diet contains 3·75 cal.

* Nutritional Biochemical Corp.
† 'Argo'.
‡ Pure vegetable fat from Procter and Gamble.
§ Fleishmann 2019, obtained from Standard Brands, Inc., New York City, New York.

Rats: Experiment 1: Adult male Sprague–Dawley rats, weighing between 460 and 510 gm., were kept in standard metabolic cages at a constant temperature of 78° F. Regular periods of light and darkness were pre-set by an automatic switch. The animals were fed diet *A* of Table 3 once daily. Feeding consisted of adding new food to the cage after transferring the amount left over from the previous feeding to a separate compartment of the feeder. Animals were thus exposed to food at all times. Urines were preserved by addition of 50 ml. of 1 *N* sulphuric acid to the container placed under the funnel-shaped bottom of the cage. Urines and fæces were collected three times a week and analysed separately. Hair and whatever nitrogen could be recovered from the cages by careful washing with distilled water were collected together with stools. Nitrogen determinations were performed as described previously[8].

Rats were distributed among three groups, receiving different amounts of nitrogen. The results are presented in Table 4.

With the cages used in this experiment, separation of stools from urine was accomplished by means of a fine-mesh screen placed between the animal and the flask where urine was collected. It was thus possible that some urine might have been absorbed by the stools. Some urea could have been converted to ammonia gas and thus have escaped collection. To obviate this possibility, and to reduce experimental errors further, another experiment with rats was carried out.

Experiment 2: Special metabolic cages were constructed. They consisted of a 'Pyrex' animal jar divided, by means of a coarse (No. 3 mesh) stainless steel screen, into an upper compartment housing the animal and a lower compartment containing 450 ml. of 1 *N* sulphuric acid. Urine, fæces and other solid and liquid matter were thus immediately collected in the lower compartment under sulphuric acid. Food was offered in a special feeder, consisting of an outer stainless steel cup covered with a screen in which was set a smaller 'Pyrex' beaker. This permitted the food spilled by the animal to be safely collected on the bottom of the outer cup.

New food was offered once a day as described for Experiment 1. Cages, feeders and screens were copiously washed and urine, fæces, spilled food, food

Table 4. Nitrogen Balances and Variations in Body-weight in Male Sprague–Dawley Rats kept at Various Nitrogen and Calorie Intakes

Group	No. of animals	Diet	Average daily intake/rat Nitrogen (mgm.)	Average daily intake/rat Calories	Consecutive days observed	Av. total nitrogen 'retention' (gm./rat)	Av. gain in body-weight (gm./rat) Init.	Expected*	Found
I	4	*A*	437	52·5	140	18 ± 3	465	+540	+5
II	7	*A*	374	45·0	140	17 ± 2	473	+510	−20
III	7	*A*	343	41·2	140	14 ± 2	468	+420	−90

* Nitrogen retained × 30.

Table 5. NITROGEN BALANCES AND VARIATIONS IN BODY-WEIGHT IN MALE SPRAGUE-DAWLEY RATS
(same rats used throughout)

No. of animals	Diet	Average weekly intake: Nitrogen (mgm.) Offered	Nitrogen (mgm.) Eaten	Calories Offered	Calories Eaten	Consecutive weeks observed	Av. total nitrogen 'retention' (mgm./rat)	Av. body-weight (gm./rat): Init.	Gain: Expected*	Gain: Found
6	*A*	6,654	5,118	787·5	615	3	+ 798	477	+23	+33
6	*B*	12,138	8,092	787·5	525	3	+1,119	510	+33	+23

* Nitrogen retained × 30.

left over and whatever could be collected by repeated washings, were pooled and analysed in a single weekly pool. Food left over was weighed before it was added to the 'cage content pool'. At the end of the washing, the cages appeared absolutely clean and no traces of solid matter could be seen.

Although this method gives no information concerning the partition of nitrogen between urine, fæces and other potential routes of elimination such as hair, nails, or saliva, it has the advantage of yielding the most accurate results concerning the nitrogen balance, because it eliminates multiple errors possible in the determination of several components rather than a single pool. Before analysis, the 'pool' was homogenized for 5 min. in a large blender and brought to volume with enough sulphuric acid to make the final concentration 30 per cent (v/v). Food was analysed in lots equal to the weekly offering, following a procedure identical to that used for the 'pool'.

In Table 5, the relationship between nitrogen intake, nitrogen 'retention' and changes in body-weight is shown for various diets and various nitrogen intakes. The results obtained with the less-critical method of collection (Table 4) are substantially confirmed. Unfortunately, the variations in body-weight are not as demonstrative as those presented in Table 4. It is clearly evident, nevertheless, that they are independent of the nitrogen retention, and most likely represent fat storage.

Mice: Experiments with mice were carried out exactly as in rat experiment 2 except for smaller metabolic cages.

In Table 6, the nitrogen balances and variations in body-weight are presented at two different levels of nitrogen intake. It is interesting to note the higher fraction of the intake 'retained' by mice as compared with rats.

Over long periods of time, our animals have 'retained' large quantities of nitrogen. In some experiments, these were in excess of the amount the whole animal could have contained at the beginning of the experiment. Nevertheless, this nitrogen 'retention' was not represented by gain in weight.

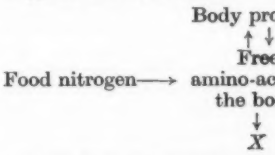
The 'retention' observed does not appear to be due to a technical artefact. This point is supported by the following considerations: The validity of the Kjeldahl method used was tested by analysing compounds the composition and purity of which were verified by independent carbon and hydrogen analysis. Twenty-five commonly occurring amino-acids and representative naturally occurring purines were analysed and gave results within 1 per cent of the theoretical value. Experiments were designed which maintained nitrogen equilibrium in dogs at suitably low nitrogen intakes, thus confirming the accuracy and completeness of our collections.

The length and magnitude of the 'retention' makes untenable the explanation that nitrogen could be stored at the expense of other constituents of the body. It should be added that, whenever tested[6,7], experimental evidence has not supported such an interpretation.

The hypothesis that some of the nitrogen fed in excess is eliminated through an unmeasured and hitherto unrecognized pathway would explain the discrepancies noted in a simple and rational way.

It does not seem likely that such a pathway could be represented by colonic flatus. Data available in man[3,9,10] show that such a pathway is possibly quantitatively insufficient as a route for loss of nitrogen. The small amounts of ammonia eliminated via respiration[14,15] would also fail to represent a quantitatively satisfactory explanation of our results.

The 'retention' occurs to a significant extent only when excess nitrogen is offered to the animal, as though such a pathway were active in providing a clearing mechanism for the nitrogen not warranted by metabolic need of the organism. This characteristic suggests the following schema:



That *X* is eliminated through respiration appears a likely possibility. If *X* exists and is represented by nitrogen gas, an amount which could explain the discrepancy between gain in body-weight and 'retention' would go undetected by the usual methods of analysis of nitrogen gas, being less than one-thousandth of the nitrogen involved in the respiration during 24 hr.

The non-participation of nitrogen in respiratory exchange has been postulated rather than proved. On the contrary, the few results available[12,15] suggest that nitrogen does participate in the respiratory exchange. The possibility that other gases in

Table 6. NITROGEN BALANCES AND VARIATIONS IN BODY-WEIGHT AT VARIOUS NITROGEN INTAKES IN MICE

No. of animals	Diet	Average weekly intake: Nitrogen (mgm.) Offered	Nitrogen (mgm.) Eaten	Calories Offered	Calories Eaten	Consecutive weeks observed	Av. total nitrogen 'retention' (mgm./mouse)	Av. body-weight (gm./mouse): Init.	Gain: Expected*	Gain: Found
5	*A*	1,210	1,170	157·5	141·0	6	492	44	+15	+1
	B	2,427	1,850	157·5	120	3	720	45	+22	0

* Nitrogen retention × 30.

addition to nitrogen and ammonia and involving nitrogen could be eliminated via respiration has not been excluded.

The presence of a metabolic route of food nitrogen different from those already known could offer an explanation for the so-called specific dynamic action of protein. It should be remembered that the specific dynamic action is shown when 'excess' protein is fed to an animal in weight-equilibrium, but is absent when the same protein, although possibly fed in 'excess', is stored in the growing organism. My schema is consistent with such findings, and could explain the production of the 'extra' heat with the production of X through a chain of metabolic steps which might well be exothermic. The fact that specific dynamic action does not occur after hepatectomy and that only a few amino-acids are capable of eliciting it under particular experimental conditions[16,17] is not inconsistent with my hypothesis.

Several anomalies of mammalian nitrogen metabolism could be explained in a simple and credible way by the loss in appreciable amounts of a nitrogen-containing metabolite, eliminated possibly through respiration. Adequate respirometers have been constructed, and an investigation involving the use of nitrogen-15 is under way in our laboratory, at the pre-clinical and clinical level, to confirm or disprove this hypothesis, with the ultimate aim of identification of the pathway and its metabolites.

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ENERGETIC ASPECTS OF THE MITOCHONDRIAL OXIDATION OF SUCCINATE

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THE reaction mechanism by which the enzymic oxidation of succinate is linked to the terminal electron transport system of intact mitochondria has been the subject of intense work during the past decade. Recent work in both the present laboratories, as well as in Chance's laboratory, has directed attention towards some new energetic aspects of this mechanism.

Prompted by an early observation of Chance and Hollunger[1], Klingenberg *et al.*[2,3], demonstrated that the addition of succinate to mitochondria, which were in a state of respiratory control ("State 4" in Chance's terminology), was followed by a considerable reduction of the intramitochondrial pyridine nucleotide. The extent of this reduction was higher than than obtained with pyridine nucleotide-linked substrates. It was postulated that this succinate-linked reduction of the mitochondrial pyridine nucleotide is an energy-requiring process, which, as recently reported by Chance and Hollunger[4], is inhibited by uncouplers of oxidative phosphorylation, and is not restored in this state by added adenosine triphosphate. It has also been shown that a similar high extent of reduction of the mitochondrial pyridine nucleotide may be obtained by another flavin-linked substrate, glycerol-1-phosphate[5,6], and that both the succinate- and the glycerol-1-phosphate-induced reduction may, in some instances, require the presence of added adenosine triphosphate[6]. In these instances also the reduction of a flavoprotein was found to be dependent on oxidative phosphorylation[6].

Recently it was reported by Azzone and Ernster[7], that the aerobic oxidation of succinate is greatly inhibited in rat liver mitochondria which had been depleted of endogenous high-energy phosphate by incubation, under appropriate conditions, with arsenate and/or agents which uncouple oxidative phosphorylation. In these mitochondria the rate of aerobic oxidation of succinate could be greatly stimulated by the addition of adenosine triphosphate, and this effect was not abolished by the simultaneous presence of uncouplers of phosphorylation. It was postulated that, under these conditions, the aerobic oxidation of succinate requires high-energy phosphate and that this energy can be supplied by added adenosine triphosphate through a reaction mechanism which is not inhibited by uncouplers of electron transport-coupled phosphorylation.

The objective of the present work was to elucidate the possible relation between the energy requirement of succinate oxidation and the energy-requiring reduction of pyridine nucleotide. In particular, it was of interest to investigate whether the adenosine triphosphate-induced stimulation of the aerobic

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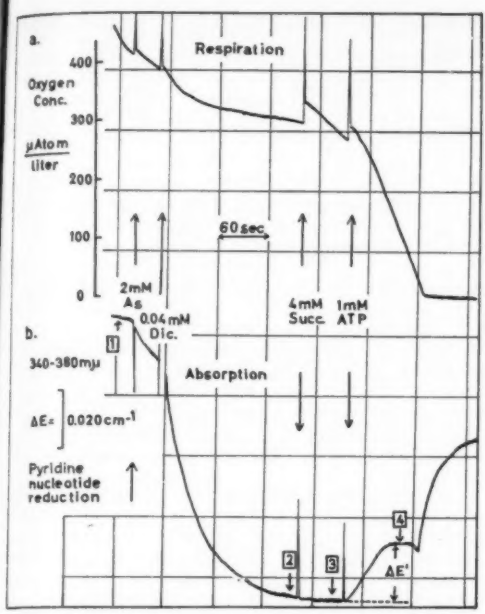

Fig. 1. Simultaneous recording of respiration (*a*) and pyridine nucleotide absorption (*b*). Rat liver mitochondria, 3·8 mgm. prot./ml. in 0·13 m*M* potassium chloride, 0·008 *M* Magnesium chlorate, 0·02 *M* triethanolamine hydrochloride, *p*H 7·5. *As*, arsenate; *Dic*, dicoumarol; *Succ*, succinate. Discontinuous absorption trace at the addition of dicoumarol because of compensation for the dicoumarol absorption. The numbers indicate the moments of extraction for pyridine nucleotide analysis, the results of which are given in Table 1

oxidation of succinate in mitochondria depleted of high-energy phosphate is accompanied by a reduction of the mitochondrial pyridine nucleotide, and whether this process, similarly to the adenosine triphosphate-induced respiratory stimulation, can take place in the presence of uncouplers. The results presented here clearly indicate that this is the case. Qualitative and quantitative estimates of the reduced pyridine nucleotides were also carried out, and, in addition, data were obtained on the oxido-reduction states of the mitochondrial flavin and cytochrome *b*. Some implications of these findings for the mechanism of the functional link of succinic dehydrogenase to the terminal electron transport system will be considered.

The experiments entailed a simultaneous measurement of the oxygen uptake and of the difference in spectral absorption at suitable wave-lengths, using a double-beam spectrophotometer equipped with a vibrating oxygen electrode[8]. Twice-washed rat liver mitochondria were prepared as previously described by Klingenberg *et al.*[3]. The incubation medium contained 0·13 *M* potassium chloride, 0·02 *M* triethanolamine buffer, *p*H 7·5, and 0·008 *M* magnesium chloride. The measurements were carried out at 25° C. in quartz cuvettes of 0·5 and 1 cm. light-path. Extractions and enzymic determinations of the reduced and oxidized pyridine nucleotides were performed essentially as described previously[2].

A typical experiment is shown in Fig. 1. In this experiment, the mitochondria were pre-incubated for 3 min. in the presence of arsenate and dicoumarol. The endogenous respiration was rather high at the onset of the pre-incubation, but declined to almost zero after about 2 min. Subsequent addition of succinate resulted in a relatively low respiration, which, however, could be greatly stimulated by the addition of adenosine triphosphate in accordance with previous findings[7]. As seen in the lower curve of Fig. 1, the 340–380 mµ absorption difference, indicative of the reduction state of the pyridine nucleotides, decreased rapidly during the initial period of the pre-incubation. This decrease levelled off concomitantly with the decline of the endogenous respiration. The addition of succinate induced no change in the steady state of the pyridine nucleotide, whereas the subsequent addition of adenosine triphosphate caused a reduction of the pyridine nucleotide. A new steady-state level was reached after about 1 min. It may be noticed that both the stimulation of the respiration and the increase of the 340–380 mµ absorption required a short lag-period, following the addition of adenosine triphosphate.

To ascertain if the spectrophotometric changes were relevant to the reduction of pyridine nucleotide, enzymic determinations were performed. For this purpose, a parallel experiment was carried out with a 10-ml. sample of the incubation mixture, from which successive aliquots were removed and extracted at the moments indicated in Fig. 1. The results of the di- and tri-phosphopyridine nucleotide determinations are given in Table 1 (*a*). The total levels of reduced pyridine nucleotide found in the various aliquots were in agreement with the spectrophotometric data. A decrease was found of the total reduced pyridine nucleotide during the pre-incubation, followed by no change on the addition of succinate, and by an increase on the addition of adenosine triphosphate.

It may also be seen in Table 1 that the adenosine triphosphate-induced reduction of the pyridine nucleotide was accounted for mainly by an increase of reduced triphosphopyridine nucleotide, and only to a small extent by an increase of reduced diphosphopyridine nucleotide. These results, which are in agreement with previous findings[2,3], indicate that in the mitochondria there occurs a transfer of hydrogen between reduced di- and tri-phosphopyridine nucleotide by way of a mechanism which favours the accumulation of the latter compound. The fact that the mitochondrial pyridine nucleotide transhydrogenase[9] cannot account for the presence of a higher level of reduced triphosphopyridine nucleotide suggests that other transhydrogenase mechanisms[10] may also be involved.

The total levels of both reduced pyridine nucleotides, attained upon the addition of adenosine

Table 1. Pyridine Nucleotide Analysis on Extracts from Liver Mitochondria

Successive additions	*T*†	DPNH µmol./gm. prot.	TPNH µmol./gm. prot.
(*a*) Arsenate-dicoumarol treatment			
—	10″ (1)*	0·78	3·60
2 m*M* arsenate			
0·04 m*M* dicoumarol	3′ 40″ (2)	0·07	0·47
4 m*M* succinate	5′ 40″ (3)	0·05	0·38
2 m*M* ATP	7′ 40″ (4)	0·15	0·83
(*b*) Dinitrophenol treatment			
—	10″	0·72	3·60
0·1 m*M* dinitrophenol	4′ 10″	0·05	0·46
4 m*M* succinate	6′ 10″	0·08	0·72
2 m*M* ATP	8′ 10″	0·15	1·37
1 m*M* KCN	13′	1·00	1·67

* Extraction indicated by the same numbers in Fig. 1*b*.
† Time of extractions after incubation.

triphosphate, were relatively low in these experiments (5–10 per cent for diphosphopyridine nucleotides and about 30 per cent for triphosphopyridine nucleotides) as compared with those previously observed[2,3] in mitochondria under respiratory control in the presence of succinate (75 and 95 per cent, respectively). However, the present levels are in fair agreement with those found[2,3] when an uncoupler of oxidative phosphorylation was added to this latter system, subsequent to the addition of succinate. These levels would therefore seem to represent the maxima obtainable in a state of uncontrolled respiration.

In Table 2, data are summarized which show the influence of various pre-incubating conditions on the phenomenon described above. Replacement of dicoumarol by dinitrophenol had no major effect on the respiratory-rate either before or after the addition of adenosine triphosphate. On the other hand, the extent of reduction of the mitochondrial pyridine nucleotide following the addition of adenosine triphosphate was lowered to about one-half.

Table 2. Effect of Adenosine Triphosphate on Succinate Respiration and Pyridine Nucleotide Reduction of Pre-incubated Liver Mitochondria

Pre-incubation with	Oxygen uptake with 4 m*M* succinate before | after addition of 2 m*M* ATP		Absorbancy increase E' at 340–380 mμ (cf. Fig. 1*b*) on addition of 2 m*M* ATP
	μ atom O / min. gm. prot.		1 / cm. gm. prot.
2 m*M* arsenate + 0·04 m*M* dicoumarol	13	55	11
2 m*M* arsenate + 0·1 m*M* DNP	12	44	5·5
0·1 m*M* DNP	20	43	5
2 m*M* arsenate	40	no ATP added (succinate alone):	30

Pre-incubation with arsenate alone resulted in a low rate of respiration with succinate, which, however, gradually increased even without the addition of adenosine triphosphate. As suggested elsewhere[11], this effect may be attributed to a resynthesis of high-energy phosphate within the mitochondria during the course of succinate oxidation. The gradual increase of the respiratory-rate was paralleled by a reduction of the pyridine nucleotide, which reached a steady-state level of about twice that attained in the presence of adenosine triphosphate in the arsenate-dicoumarol-pretreated system.

When the mitochondria were pre-incubated with dinitrophenol (or dicoumarol) in the absence of arsenate, an inhibition of the subsequent respiration with succinate was also obtained, although the inhibition was weaker than in the simultaneous presence of arsenate. The rate of the inhibited respiration, however, was constant, exhibiting no tendency to increase without the addition of adenosine triphosphate as in the case of pre-treatment with arsenate alone. Adenosine triphosphate gave a stimulation of the respiration in this case also, and again, this effect was accompanied by a reduction of the pyridine nucleotide. The steady-state level reached in the presence of dinitrophenol alone was about the same as in the simultaneous presence of arsenate. Enzymic determinations on the reduced pyridine nucleotide levels in the dinitrophenol system are shown in Table 1 (*b*).

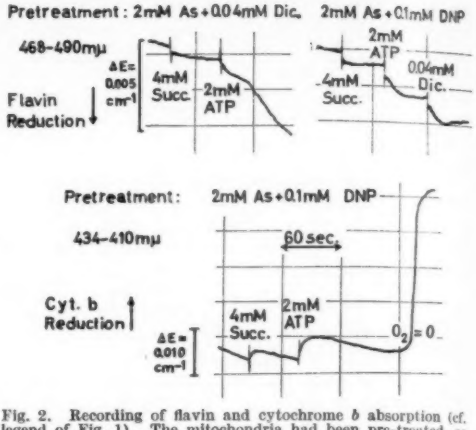

Fig. 2. Recording of flavin and cytochrome *b* absorption (cf. legend of Fig. 1). The mitochondria had been pre-treated, as indicated, for 3 min. until succinate addition

The difference in findings with dinitrophenol alone and arsenate alone as pre-incubating agents may be ascribed to the fact that although arsenate is very efficient in depleting mitochondria of high-energy phosphate it is a weak uncoupler of electron transport-coupled phosphorylation, whereas dinitrophenol is a potent uncoupler, but rather poor in depleting the mitochondria of high-energy phosphate. As shown elsewhere[11], dinitrophenol can efficiently deplete mitochondria of high-energy phosphate in the presence of adenosine-5′-phosphate.

Under all the conditions tested in Table 2, the rate of reduction of the pyridine nucleotide was approximately the same.

In other experiments, the changes in oxidation-reduction states of the mitochondrial flavin and cytochrome *b* were followed (Fig. 2). Increased levels of reduction of both these components were recorded following the stimulation of succinate oxidation by the addition of adenosine triphosphate in mitochondria that were depleted of high-energy phosphate.

A difference in the extent and rate of the adenosine triphosphate-induced reduction of the flavin was observed according to whether dinitrophenol or dicoumarol was used as a pre-incubating agent in combination with arsenate. In the case of dicoumarol, the change in the steady state of the flavin was diphasic and the amount of flavin reduced was about twice that obtained with dinitrophenol. As was observed above (Table 2), the reduced pyridine nucleotide-level was also about twice or higher in the dicoumarol system than that attained with the dinitrophenol system. These findings may be related to the observation previously reported that dicoumarol, besides its uncoupling effect, is a powerful inhibitor of a soluble, pyridine nucleotide-unspecific diaphorase which is also found in mitochondria[12]. In support of this concept, addition of both dinitrophenol and dicoumarol to the present system resulted in an adenosine triphosphate-induced reduction of flavin of the same rate and extent as that obtained in the presence of dicoumarol alone.

The possibility that oxaloacetate was responsible for the observed inhibition of succinate oxidation after the arsenate-dicoumarol incubation was previously[7] discarded on the evidence that cysteine sulphinic acid, which removes oxaloacetate by trans-

amination to aspartate, could not restore succinate oxidation. This question was re-investigated in the present work by measuring directly the oxaloacetate concentration after the arsenate–dicoumarol pre-incubation. In no case was it possible to find oxaloacetate in the incubation mixture in amounts exceeding the sensitivity of the method used; that is, 5×10^{-8} *M*. Furthermore, since it has been demonstrated that adenosine triphosphate can remove the oxaloacetic inhibition by formation of phosphoenol-pyruvate[12], this compound also was determined. The concentration of phosphoenol-pyruvate was ascertained to be less than 5×10^{-7} *M* and thus cannot account for the observed adenosine triphosphate effect.

The present findings are at some variance with those recently reported by Chance and Hollunger[4]. These authors found no increase in the reduction of pyridine nucleotides following the addition of adenosine triphosphate in the presence of even very low concentrations of uncouplers. In the present case, added adenosine triphosphate clearly was able to induce a reduction of the pyridine nucleotide in the presence of concentrations of dinitrophenol or dicoumarol, which fully uncouple oxidative phosphorylation. It would seem, however, that, although the experiments of Chance and Hollunger, and those in the present work, were made with mitochondria of very different initial functional states, the discrepancy in the findings may not be due to different reaction mechanisms, but rather to a possible difference in the suitability of the two sets of conditions for allowing this adenosine triphosphate effect to become manifest.

The results presented here indicate a striking parallelism between the effect of adenosine triphosphate on respiration and on pyridine nucleotide reduction, and therefore suggest that under the present conditions the energy requirement for the two processes is utilized through a common intermediate. It would also seem that the common high-energy intermediate, which is formed during the oxidation of succinate, and the energy of which may be utilized for pyridine nucleotide reduction, is situated in the initial part of the respiratory chain, thus being probably closely related to the primary high-energy intermediate involved in the first respiratory chain phosphorylation. Furthermore, since the need for energy to initiate succinate oxidation is thought to be a normal property of intact mitochondria, it follows from the above reasoning that the site of entrance of electrons from succinic dehydrogenase to the mitochondrial respiratory chain should be prior to the level of cytochrome *b*; otherwise, the aerobic oxidation of succinate could not result normally in a phosphate/oxygen ratio of 2.

From the fact that concentrations of dinitrophenol which uncouple oxidative phosphorylation do not abolish the stimulatory effects of adenosine triphosphate on succinate-oxidation and on reduction of pyridine nucleotide by succinate, it appears that energy of adenosine triphosphate is available to the respiratory chain in spite of the presence of uncouplers of oxidative phosphorylation. This observation seems scarcely compatible with those hypotheses of oxidative phosphorylation[14] in which a non-phosphorylated high-energy intermediate, susceptible to hydrolysis in the presence of dinitrophenol, is involved between adenosine triphosphate and the respiratory chain. On the other hand, these results are consistent with those hypotheses which postulate the formation of a high-energy phosphate compound involving a respiratory carrier such as flavin[15], or a quinone[16], as primary intermediate of electron transport coupled phosphorylation. As has been pointed out previously[15], in this type of hypothesis dinitrophenol is supposed to act by interfering with the activation of inorganic orthophosphate, thus inhibiting only the forward, but not the backward, reaction of respiratory chain phosphorylations.

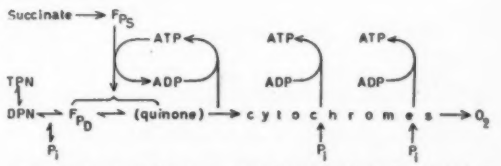

Fig. 3. Hypothetical scheme of the functional link of succinic dehydrogenase to the terminal electron transport system. Symbols: DPN, diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide; *Fp*$_D$, DPN diaphorase; *Fps*, succinic dehydrogenase; Pi, inorganic phosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate

Fig. 3 shows a tentative scheme covering these conclusions. It is suggested that the electrons from reduced succinic dehydrogenase enter the respiratory chain at the level of the diaphorase flavin, or alternatively, at the level of a quinone situated between the diaphorase flavin and cytochrome *b*. In order to react with these carriers the reduced succinic dehydrogenase requires the intervention of high-energy phosphate, with the reduced phosphorylated diaphorase or quinone as the reaction product. This product can react either with cytochrome *b*, resulting in the regeneration of adenosine triphosphate, or with diphosphopyridine nucleotide, with the liberation of orthophosphate.

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PREPARATION AND IMMUNOLOGICAL PROPERTIES OF AN ARTIFICIAL ANTIGEN WITH COLITOSE (3-DEOXY-L-FUCOSE) AS THE DETERMINANT GROUP

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ANTIGENS are principally substances of high molecular weight of which only a small part, the determinant group(s), is responsible for specificity[1,2]. Most of our knowledge of natural antigens originates from immunochemical work on bacterial polysaccharides. Inhibition of the specific precipitation of bacterial polysaccharides with their respective antibodies by oligosaccharides of low molecular weight has demonstrated the important role of sugar constituents situated at the terminal non-reducing end of side-chains[3,4].

In the course of immunochemical work on *Salmonella*, *Escherichia* and other Enterobacteriaceae the terminal sugars of determinant groups of various *O* antigenic polysaccharides were recognized[5–8]. It was found that terminally linked colitose (3-deoxy-L-fucose)[9] functions as the determinant end-group of the *O* antigen of *E. coli O* 111 *B* 4 and of the cross-reacting *O* antigens of *Salmonella O* 35 (ref. 6) and of *Arizona O* 20 (refs. 10 and 11). It is further known that another group of cross-reacting enterobacterial organisms exists, namely, *E. coli O* 55 *B* 5, *Salmonella O* 50 and *Arizona O* 9, the *O* antigens of which also contain terminally linked colitose[10]. Using standard rabbit antisera, the *E. coli O* 111 (with *Salmonella O* 35, *Arizona O* 20) group on one hand, and the *E. coli O* 55 (with *Salmonella O* 50, *Arizona O* 9) group on the other, do not show any overlapping cross-reactivity. It was therefore concluded that colitose in the *O* antigens of both cross-reacting groups must be linked in a different manner, leading to different kinds of rabbit antibodies. It is, however, possible to obtain immune sera reacting with the *O* antigens of both groups after immunization of animal species other than the rabbit, such as horses, cows or goats. With horse immune sera it was shown that cross-reactions between *O* antigens of the *E. coli O* 111 and *E. coli O* 55 groups were in fact due to determinant groups both of which contained terminal colitose[6].

For the further elucidation of the role of colitose as the determinant end-group in bacterial polysaccharides artificial antigens containing colitose linked to a protein have been prepared. Antisera to these colitose-antigens were examined for possible cross-reactions with bacterial *O* antigens, containing terminal colitose, and with the corresponding micro-organisms.

Preparation of the colitose-antigens. Artificial antigens were prepared by coupling diazotized *p*-aminophenyl-α- and -β-colito-pyranosides to proteins according to the classical azo-method of K. Landsteiner (see refs. 1 and 2). Colitose[9] was acetylated and the resulting mixture of the α- and β-triacetates was heated with *p*-nitrophenol in the presence of mercuric chloride under reduced pressure[12,13]. A mixture was obtained in good yield of *p*-nitrophenyl-diacetyl-α- and β-colitopyranosides. On recrystallization from methanol the predominating and less soluble α-form was obtained in pure form (m.p. 180°; $[\alpha]_D^{20}$ − 213° (methanol)). The β-derivative, slightly contaminated by the α-form, was isolated from the mother liquor (m.p. 155°; $[\alpha]_D^{20}$ −0° (methanol)). After deacetylation the *p*-nitrophenyl-colitosides were converted into the corresponding *p*-aminophenyl-α- and β-colitosides by catalytic hydrogenation: *p*-aminophenyl-α-colitopyranoside: m.p. 180°, $[\alpha]_D^{20}$ − 166° (methanol); calc.: C, 60·2; H, 7·1; N, 5·8; found: C, 60·4; H, 7·1; N, 5·8. *p*-Aminophenyl-β-colitopyranoside: m.p. 75°, $[\alpha]_D^{20}$ + 99° (methanol). The β-isomer may still contain some α-form. For the preparation of the colito-phenylazoproteins the *p*-aminophenyl-colitosides were diazotized and added to alkaline solutions of purified bovine serum albumin or egg albumin. The red-coloured solutions of the azoproteins were dialysed, adjusted to the desired concentration and kept sterile with merthiolate (1 : 10,000). Antigen solutions contained about 1 mgm. of bound colitose and 16 mgm. of protein per ml. On the average, one molecule of serum albumin contained about 30 colito-phenylazo groups.

Colito-pyranosido-phenylazo-protein

The following artificial antigens were prepared:

α-colitose phenylazo-bovalbumin	= α-*Co-Bo*
α-colitose phenylazo-ovalbumin	= α-*Co-Ov*
β-colitose phenylazo-bovalbumin	= β-*Co-Bo*
β-colitose phenylazo-ovalbumin	= β-*Co-Ov*

Reaction of the artificial antigens and haptens with horse anti E. coli *sera.* Precipitation of *E. coli O*111 polysaccharide of a horse anti *E. coli O* 111 serum was inhibited by various preparations in the following order: α-aminophenyl-colito-pyranoside > β-amino-

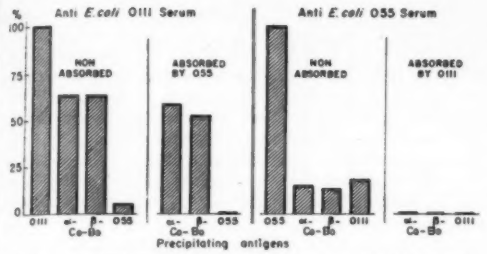

Fig. 1. Per cent precipitation of horse anti *E. coli*. *O* 111 and anti *E. coli O* 55 antibodies by artificial colitose antigens *O* 111 = Polysaccharide extracted from *E. coli O* 111; *O* 55 = polysaccharide extracted from *E. coli O* 55; α- and β-*Co-Bo* = α- and β-colitose phenylazo-bovalbumin

phenyl-colito-pyranoside > colitose. In the *E. coli O* 111 antigen the immunologically determinant colitose end-groups must therefore be linked as colito-pyranoside. It is not known at present whether or not the relatively strong inhibition of the β-isomer, as compared to colitose, is due to contamination with small amounts of the α-isomer.

The four colitose phenylazo-proteins gave strong precipitation with a horse anti *E. coli O* 111 serum and a weaker precipitation with a horse anti *E. coli O* 55 serum. Absorption of each of the antisera with the respective homologous polysaccharide markedly diminished the precipitating antibodies towards the artificial antigens. Moreover, after absorption of the antisera with the heterologous cross-reacting polysaccharide, precipitation of the anti *E. coli O* 111 serum with the artificial colitose antigens was only slightly diminished, whereas precipitation of the anti *E. coli O* 55 serum with the artificial antigens was completely abolished, as shown in Fig. 1. This suggests that in horse anti *O* 55 serum the small proportion of antibodies which reacts with the artificial colitose antigens is only contained in the antibodies cross-reacting with *E. coli O* 111 polysaccharide. In these experiments no difference in serological behaviour was found between the α- and β-colitosides.

Immunization of animals with artificial colitose-antigens. Rabbits were injected with each of the colitose-phenylazo-proteins. In nine out of thirteen sera precipitins against the artificial α- or β-colitose antigens were developed during immunization. Some of the precipitations were very strong. Inhibition by colitose and aminophenyl-colitosides indicated that these precipitations were due mostly, if not completely, to anti-colitose antibodies. Bacterial colitose-containing polysaccharides were not precipitated by anti-colitose rabbit sera. Two sera agglutinated *E. coli O* 111 suspensions as well as erythrocytes sensitized with the corresponding lipopolysaccharide[14,15]. However, the titres were relatively low, and no correlation was found with the levels of anti-colitose antibodies.

One goat (goat 1) was immunized with α-*Co-Bo* and a second goat (goat 2) with β-*Co-Bo*. The antisera obtained gave precipitin reactions with colitose-ovalbumin antigens (α-*Co-Ov* and β-*Co-Ov*), showing that specific anti-colitose antibodies had been formed. In addition, the sera were found to contain antibodies which specifically cross-reacted with *E. coli O* 111, that is, they precipitated *E. coli O* 111 polysaccharide; erythrocytes sensitized with *E. coli O* 111 lipopolysaccharide and suspensions of heated *E. coli O* 111 organisms were agglutinated, as shown in Table 1. On the other hand, the sera did not react with *E. coli O* 55 polysaccharide (no precipitation, no hæmagglutination), but one of the two sera agglutinated suspensions of *E. coli O* 55 organisms (goat 1). This antiserum behaved exactly like an anti *E. coli O* 111 horse or goat serum. *E. coli O* 111 polysaccharide removed all agglutinating antibodies from this serum. Furthermore, in hæmagglutination tests the two sera cross-reacted with all bacterial species serologically related to *E. coli O* 111 (*Salmonella O* 35, *Arizona O* 20), but not with species serologically related to *E. coli O* 55 (*Salmonella O* 50, *Arizona O* 9). The goat sera before immunization showed low titres of natural antibodies against practically all tested enterobacterial *O* antigens. After immunization with the artificial colitose-antigens only, titres against *E. coli O* 111 and serologically related strains were found to be markedly elevated (Table 1).

Twenty years ago W. F. Goebel[16,17] described the isolation of cellobiuronic acid as the determinant group of the capsular polysaccharide antigen of *Pneumococcus* type III and the synthesis of an artificial antigen by coupling diazotized *p*-aminobenzyl cellobiuronide to protein. The antisera so obtained strongly precipitated type III polysaccharide and agglutinated type III *Pneumococci*. Furthermore, they protected animals against otherwise fatal infections with virulent cultures of these Gram-

Table 1: Antimicrobial Antibody Titres of Goat Sera before and after Immunization with Artificial Antigens (α- and β-*Co-Bo*)

Antigen (Bacteria or lipopolysacharides) used		Goat 1 (immunized with α-*Co-Bo*)				Goat 2 (immunized with β-*Co-Bo*)			
		Hæmagglutination*		Agglutination†		Hæmagglutination*		Agglutination†	
		before	after	before	after	before	after	before	after
(*a*) *E. coli*	*O* 111 *B4*	320	5,120	<100	800	80	2,560	40	800
(*b*) ,,	*O* 55 *B5*	80	80	<100	400	80	80	40	80
(*c*) ,,	*O* 26	20	20	320	400	40	40	160	100
(*c*) ,,	*O* 86	20	20	—	—	80	40	—	—
(*c*) ,,	*O* 8	80	40	—	—	80	40	—	—
(*a*) *S. adelaide*	*O* 35	—	—	20	400	—	—	20	320
(*a*) *S. monschaui*	*O* 35	40	2,560	—	—	40	1,280	—	—
(*c*) *S. cholerae suis*	*O* 6/7	<10	<10	<100	<100	160	40	—	—
(*b*) *S. greenside*	*O* 50	—	—	40	400	—	—	40	40
(*c*) *S. abortus equi*	*O* 4/12	10	10	20	<100	10	10	—	—
(*b*) Arizona	*O* 9*a*	—	—	40	200	—	—	20	40
(*a*) Arizona	*O* 20	—	—	—	—	—	—	20	320

* Hæmagglutination of erythrocytes sensitized with lipopolysaccharides (see ref. 14). † Agglutination of heated suspensions

All strains (*a*) contain colitose and cross-react. All strains (*b*) contain colitose and cross-react but not with strains (*a*). All strains (*c*) do not contain colitose and do not cross-react either between them or with strains (*a*) and (*b*).

positive organisms. The present article reports the first artificial antigen which stimulates antibodies against certain pathogenic Gram-negative bacteria.

In agreement with previous inhibition work[6], experiments described here show that a single monosaccharide unit—colitose—suffices to account to a high degree for the antigenic specificity of *E. coli O* 111 and serologically related bacteria (*Salmonella O* 35, *Arizona O* 20).

Rabbit antisera prepared against artificial colitose antigens were frequently found to be rich in specific anticolitose antibodies; they reacted only weakly, if at all, with the microbial colitose-containing polysaccharides. On the other hand, the two goat sera reacted strongly with the microbial polysaccharides although they showed lower titres of precipitating anticolitose antibodies, as compared with the rabbit sera. The quality of the antibodies obtained from the two species is obviously very different. This is a further example of the significance of the species in immunization with respect to the specificity of antibodies obtained by one and the same antigen.

Only one group of bacterial polysaccharides containing terminal colitose (*E. coli O* 111, *Salmonella O* 35, *Arizona O* 20) cross-reacted with goat antisera to the artificial colitose antigens, in perfect agreement with the finding that only anti *E. coli O* 111 antibodies precipitate colitose phenylazo-proteins. In the polysaccharide antigens of the *E. coli O* 111 group the structural arrangement of the terminally linked colitose appears to be similar to that of colitose in the artificial colitose antigens. On the other hand, on the basis of the findings reported here, the status of the likewise terminally bound colitose in the polysaccharides of the *E. coli O* 55 group (*E. coli O* 55, *Salmonella O* 50, Arizona *O* 9) has again been shown to be different[9,10].

It should be pointed out that, in contrast to the bacterial endotoxic *O* antigen complexes, the artificial colitose antigens are practically non-toxic.

Work on the biological properties of anticolitose goat sera is in progress. Anticolitose sera from other animal species will be examined. The preparation of other 3,6-dideoxy-hexose phenylazo-proteins and investigations of their immunological properties are also under way.

Experimental details will be given elsewhere.

We are grateful to Prof. F. Kauffmann, Statens Seruminstitut, Copenhagen, for discussing the manuscript and for confirming the hæm agglutination experiments with a goat anti-colitose serum.

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OCCURRENCE OF D-PHENYLALANINE, D-ALLOTHREONINE AND OTHER D-AMINO-ACIDS IN PEPTIDO-LIPIDS OF BACTERIAL ORIGIN

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WORK on the chemical constitution of antibiotics has shown that a large number of these substances contain amino-acids of the D-configuration[1,2]. The occurrence of D-amino-acids in functional structures of living cells has been reported much more rarely. However, D-alanine, D-glutamic acid, and D-aspartic acid have been shown recently to be common and major constituents of the bacterial cell wall[3–7], and D-alanine and D-glutamic acid have been found to be present in the 'spore peptide' of *Bacillus megaterium*[8]. Moreover, α,ε-diaminopimelic acid, a major constituent of many bacterial cell walls[9], possesses the *meso*-configuration for a large number of micro-organisms[10]. Certain nucleotides exemplified by those that accumulate in penicillin-treated *Staphylococcus aureus*[11], and which afterwards have been implicated as probable precursors in the biosynthesis of cell walls[12] also contain D-amino-acids. In all the above instances the D-amino-acids occur in products of microbiological origin. The occurrence of these compounds in higher plants and animals seems, from reports to date, to be a rarer event. Recently, however, D-serine has been found in lombricine isolated from the earthworm, *Lumbricus terrestris*[13].

We wish to describe here the general occurrence of several different D-amino acids in peptido-lipids and peptido-glyco-lipids from bacteria of the order Actinomycetales[14].

The general approach has been to separate individual amino-acids by column chromatography, and then to compare the analytical values obtained through application of the ninhydrin method with those obtained by optically specific microbiological assay methods. Major discrepancies between the two methods of analysis have proved very useful in pointing out the presence of 'unnatural' forms of certain of the amino-acids, the presence of which was then confirmed by other methods. The substances included in this work are described briefly below.

(1) 'Wax *D*' of *Mycobacterium tuberculosis* var. *hominis* (Brévannes). The purification and composition of this material has been previously described[15]. The amino-acid components were reported

Table 1. NITROGENOUS COMPONENTS OF THE PEPTIDO-LIPIDS AND PEPTIDO-GLYCO-LIPIDS OF *Mycobacterium* AND *Nocardia* SPECIES

Component	Method (see footnotes)	'Wax *D*' of *M. tuberculosis*	Mycoside *C* of *M. avium*		Mycoside *C* of *M. marianum*	Peptido-lipid of *N. asteroides*	
			Jav 5	*Jav* 4		I	II
		Total μmoles					
Alanine, total	(*a*)	176	13·1	5·05	8·8	31·5	36·5
Alanine, total	(*b*)	155	13·5	4·3	8·3	29·6	32·0
Alanine, D	(*c*)	51	13·5	5·25	9·6	20·9	19·9
'Threonine', total	(*a*)		11·0	4·75	8·9	32·7	34·2
Threonine, L	(*d*)		< 0·5	< 0·4	< 0·3	34·8	35·0
Allothreonine, D	(*c*)		11·5	5·4	8·7		
Phenylalanine, total	(*a*)		7·8	4·4			
Phenylalanine, L	(*d*)		1·6	0·7	0·07		
Phenylalanine, D	(*c*)		5·4	3·0	1·65		
Valine, L	(*d*)					27·3 (*i*)	25·0 (*i*)
Valine, D	(*c*)					< 1·3	< 0·7
Proline, total	(*a*)					17·25	17·25
Proline, L	(*d*)					16·2	16·8
Leucine-*iso*leucine, total	(*a*)					19·9 (*j*)	19·8 (*j*)
Leucine-*iso*leucine, D	(*c*)					13·3 (*j*)	13·4 (*j*)
Leucine, L	(*d*)					< 1	< 1
*Iso*leucine, L	(*d*)					1·0	0·9
Glutamic acid, total	(*a*)	105					
Glutamic acid, total	(*e*)	109					
Glutamic acid, L	(*f*)	24					
Diaminopimelic acid	(*a*)	84					
Glucosamine	(*a*)	58					
Ammonia	(*g*)	332	3·5 (*h*)	1·5	1·9	7·2	7·7

(*a*) Colorimetric ninhydrin method (ref. 21).
(*b*) Microbiological assay with *Leuconostoc citrovorum* (ref. 22).
(*c*) D-Amino-acid oxidase assay. An active enzyme preparation was obtained by extraction of acetone-dried sheep kidney powder with 0·017 *M* pH 8·3 pyrophosphate buffer (ref. 23) followed by high-speed centrifugation. To the test sample in 1·3 ml. volume in water were added 1 ml. of 0·1 *M* pH 8·3 pyrophosphate buffer and 0·2 ml. enzyme and the mixture incubated at 37° with shaking for 1 hr. One ml. of 0·1 per cent 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid was then added, the mixture allowed to stand at room temperature for 15 min., 5 ml. of 2·5 *N* sodium hydroxide added, and the optical density at 520 mμ. read after 10 min. (ref. 24). The appropriate D-amino-acid was used as the standard. Control experiments showed no oxidation of the L-amino-acids.
(*d*) Microbiological assay with *Leuconostoc mesenteroides* (ref. 25).
(*e*) Microbiological assay as described in ref. 25, but using *Lactobacillus pentosus* 124-2 as test organism. Turbidities were read after 48–72 hr. of incubation.
(*f*) Microbiological assay (ref. 25) but using *Streptococcus faecalis R* as test organism.
(*g*) Direct Nesslerization of the ammonia fraction from the 'Dowex-50' columns with modified Nessler's reagent (ref. 26).
(*h*) Glycine (6 μmoles) and the following amino-acids of undetermined configurations were also detected; serine-threonine, 41 μmoles; valine, 8 μmoles; leucine-*iso*leucine, trace.
(*i*) The valine fraction also contained α-aminobutyric acid (*c.* 3 μmoles). Since this fraction was inert on treatment with D-amino-acid oxidase, it probably contains only L-α-aminobutyric acid.
(*j*) The possible presence of D- and/or L-allo*iso*leucine has not as yet been excluded.

to be *meso*-diaminopimelic acid, alanine, of which at least one-third was L, and glutamic acid, of which at least 85 per cent was D.

(2) Mycoside *C* of *Mycobacterium avium*. The mycosides, a new class of type-specific glycolipids of Mycobacteria, have recently been described[16]. The results reported here include two mycoside *C* fractions *Jav* 5 and *Jav* 4. *Jav* 5 was eluted from a silicic acid column by chloroform–methanol (95 : 5) and had the following properties: m.p. 210°, $[\alpha]_D = -34°$ ($c = 2·0$ per cent in chloroform). The analysis was: C, 62·16; H, 8·76; N, 4·35; OCH_3, 5·52; acetyl, 5·50 per cent. ($C_{86}H_{137}N_5O_{34}$ requires: C, 61·84, H, 8·89, N, 4·51, 3 OCH_3, 5·86, 2 acetyl, 5·54). *Jav* 5 appears to be a pentapeptide linked to a hydroxy acid of approximate composition $C_{32}H_{44}O_3$, the whole being linked to 3 molecules of partially O-methylated 6-deoxyhexoses containing two O-acetyl groups[17]. *Jav* 4 was isolated from the same extract as *Jav* 5 by elution from silicic acid with chloroform–methanol (94 : 6) and had the following properties; m.p. > 200°, $[\alpha]_D = -44°$ ($c = 1·9$ per cent in chloroform). The per cent analysis was: C, 58·68; H, 8·57; N, 3·82; OCH_3, 6·62; acetyl, 6·90 ($C_{78}H_{121}N_5O_{28}$ requires: C, 59·07; H, 8·35; N, 4·48; 3 OCH_3, 5·87; 2 acetyl, 5·43). The probable structure of *Jav* 4 is a pentapeptide linked to a hydroxy acid of approximate composition $C_{24}H_{48}O_3$, the whole being linked to 4 molecules of partially O-methylated 6-deoxyhexoses containing two O-acetyl groups[17].

(3) Mycoside *C* of *Mycobacterium marianum*. This substance (m.p. 198–200°, $[\alpha]_D = -31°$ in chloroform, C, 64·65; H, 8·52; N, 4·70; OCH_3 5·02; acetyl, 6·53 per cent) was isolated by Michel and Chaput[18] (Laboratory of Biochemistry, Faculté Catholique, Lyon) from a strain isolated by Sister Marie-Suzanne and Penso[19] from a leproma and now classified as a non-photochromogenic or avian strain.

(4) Peptido-lipid of *Nocardia asteroides*. Results are given for two separate preparations of the peptido-lipid of this organism[20].

Each sample was hydrolysed in 6 *N* hydrochloric acid at 105° for 24 hr. After evaporation to dryness *in vacuo*, the hydrolysate was dissolved in a small amount of water and placed on a 'Dowex-50 (H^+)' column (9 × 850 mm., 200–400 mesh). The column was developed successively with 1, 2·5, and 4 *N* hydrochloric acid[4], and the effluent collected in 5-ml. fractions. Aliquots of each fraction were analysed by the ninhydrin method of Yemm and Cocking[21]. The ninhydrin-positive fractions were concentrated *in vacuo* to dryness and the residues dissolved in 5 ml. of water and analysed. The results are given in Table 1.

Table 2. PAPER CHROMATOGRAPHY OF THE 'THREONINE' FRACTIONS OF THE MYCOSIDES OF *M. avium* AND *M. marianum*

Compound	*R** (allothreonine)
DL-Allothreonine	1·00
Jav 4 'threonine'	0·99
Jav 5 'threonine'	0·99
M. marianum 'threonine'	0·97
DL-Threonine	1·39
DL-Serine	0·62

* Solvent system: upper phase of *n*-butanol : water : acetone : concentrated ammonia (200 : 150 : 25 : 25) (ref. 27). Due to variability in absolute R_F values, values are given relative to allothreonine. R_F (allothreonine) = 0·11–0·13

The 'threonine' fractions from the 'Dowex-50' chromatography of the hydrolysates of the mycosides of *M. avium* and *M. marianum* were chromatographed on paper and showed only a single ninhydrin-reacting spot corresponding in position to allothreonine (Table 2) and no serine or threonine. Since D-allothreonine is attacked by D-amino-acid oxidase[18], the configuration of the allothreonine was determined by this method (Table 1). That the substance was eluted from the column in the correct position for allothreonine was demonstrated by the overlapping emergence of threonine and allothreonine, the former slightly preceding the latter, when a mixture of these compounds was chromatographed on a 'Dowex-50' column and then eluted with 1 *N* hydrochloric acid.

The results demonstrate the widespread occurrence of D-amino-acids in the peptido-lipids and peptido-glyco-lipids of members of the order Actinomycetales. The alanine of 'Wax *D*' of *M. tuberculosis* var. *hominis* was found to be 1/3 D and the glutamic acid 78 per cent D, values essentially in agreement with those previously reported[15]. It is probable that the glutamic acid of this substance is completely of the D-configuration and that a certain amount of L-glutamic acid may have arisen from racemization during hydrolysis[4]. The mycosides of *M. avium* and *M. marianum* are unusual in that all the amino-acid components (alanine, allothreonine, and phenylalanine) possess the D-configuration. An additional unusual feature in these substances is the occurrence of D-allothreonine, a compound not hitherto reported in a natural product. D-Allothreonine would result from racemization of the α-carbon atom of L-threonine, and this may be the pathway by which it is formed by the micro-organism. The peptido-lipids of *N. asteroides* differ considerably in composition from the mycosides. They contain threonine, valine and proline, all of the usual L-configuration, and alanine, which appears to be 2/3 D. The composition with regard to leucine and *iso*leucine has not been completely clarified. It is clear that there are only negligible amounts of L-leucine and of L-*iso*leucine present. The discrepancy between total leucine plus *iso*leucine as determined by ninhydrin and D-leucine plus D-*iso*leucine as determined by D-amino-acid oxidase would suggest the presence of L-allo*iso*leucine. These results also do not exclude the possibility of the presence of D-allo*iso*-leucine. Experiments to clarify this point are in progress.

We (M. I. and E. E. S.) wish to acknowledge the technical assistance of Mrs. Geraldine Tomlinson and Miss Helene Schenker, and support (in part) by a grant (*E*-1575 and 3134) from the United States Public Health Service. The work of the French group has been supported (in part) by a grant (*E* 28–39) from the National Institute of Allergy and Infectious Diseases, United States Public Health Service.

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NEW PATHWAYS IN THE OXIDATIVE METABOLISM OF AROMATIC COMPOUNDS BY MICRO-ORGANISMS

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THERE exists a restricted number of pathways open for the breakdown of naturally occurring aromatic amino-acids in mammalian metabolism; inherited blocks in these have greatly influenced the development of our concepts of biochemical genetics[1].

Micro-organisms, however, are the main agents which release organic carbon locked up in benzenoid structures by the plant world. Although they display a notorious versatility in this respect, only two methods of cleavage of the aromatic nucleus by microbial enzymes have been clearly demonstrated, until very recently. These are: (1) Oxidative fission of the bond between carbon atoms bearing the hydroxyl groups of an *o*-dihydroxyphenol (for example, Fig. 1*a* and *b*): catechol → *cis-cis*-muconic acid by pyrocatechase[2,3]; protocatechuic acid →

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cis-cis-β-carboxymuconic acid through the action of protocatechuic acid oxidase[4,5]. β-Oxoadipic acid is eventually produced in both these cases[6,7], which is then enzymically split to acetyl-coenzyme *A* and succinate, to be metabolized by reactions of the tricarboxylic acid cycle[8,9]. This ring cleavage mechanism appears to play little or no part in mammalian metabolism. (2) Rupture of the bond between the carbon atom bearing a hydroxyl and an adjacent carbon atom carrying a carbon side-chain or carboxyl (for example, Fig. 2): homogentisic acid → maleyl-acetoacetate by homogentisicase[10,11]; gentisic acid → maleyl-pyruvate by gentisicase[12–14]. This method is common to micro-organisms and mammals.

It appeared until recently that the main problems of interest in the catabolism of aromatic compounds by the lower organisms were those concerned with unexplored reaction sequences leading to the already known modes of ring-fission. Work at Leeds and Bangor has now made it clear that this view is much too limited; the established mechanisms of disruption of the aromatic ring, and subsequent reactions leading from the benzene nucleus to the citric acid-cycle, are by no means ubiquitous in their occurrence. In many soil bacteria, pathways of dissimilation of aromatic compounds exist which are radically different from those previously described.

Furthermore, the clear separation, as in Fig. 1*a* and *b*, of known channels cannot now be justified for all species of microbes, for example, enzyme systems from *Neurospora crassa*[5], some *Pseudomonads*[4], a *Vibrio* (0/1)[15], and *Nocardia*[16], all split protocatechuic acid to *cis-cis*-β-carboxymuconic acid from which they afterwards produce β-oxoadipic acid, but the intermediary steps employed may differ somewhat. Moreover, the preliminary steps by which various aromatic compounds are converted to the compound which undergoes fission (that is, catechol, or protocatechuic acid, etc.) also prove to be more diverse than hitherto suspected, since an anaerobic decarboxylase has been discovered in some organisms[17,18], whereby a carboxyl group attached to the benzene nucleus is replaced by hydrogen.

Throughout this work, summarized here, both departments have benefited by a free exchange of information and biochemicals.

New Modes of Aromatic Ring-fission by Bacteria

(1) *Cleavage of protocatechuic acid by a new enzyme —'protocatechuic acid 4 : 5-oxygenase'*: Dagley and Patel[19] disrupted cells of a *Pseudomonas* grown with *p*-cresol as carbon source; the cell-free extract oxidized each mole of protocatechuate with the uptake of 1 mole of oxygen, but no carbon dioxide was liberated. Similar extracts of *Vibrio* 0/1[15] also oxidized protocatechuate; 1 mole of oxygen was utilized, 1 mole of carbon dioxide was evolved, and β-oxoadipate was produced. In the case of the *Pseudomonas* system the product was a white, crystalline, dibasic acid, m.p. 235°, shown by Trippett, Dagley and Stopher[20] to be 2 : 4-lutidinic acid. The formation of the nucleus of pyridine as a consequence of oxidizing that of benzene was surprising; the realization that it had occurred was delayed by the coincidence that the percentage of carbon and hydrogen in lutidinic acid monohydrate is practically

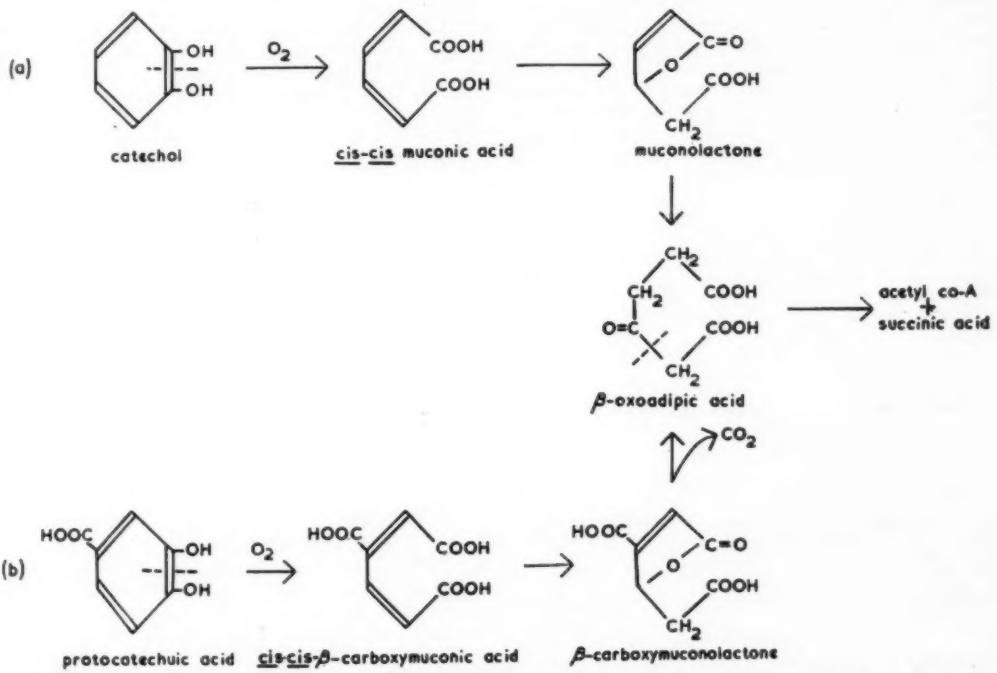

Fig. 1. Oxidative fission of o-dihydroxyphenols by some micro-organisms. *a*, Pathway of dissimilation of catechol by some *Pseudomonads*; *b*, pathway of dissimilation of protocatechuate by *Neurospora crassa*

the same as that for a compound formed from 1 mole of protocatechuic acid and 1 mole of oxygen. Stopher *et al.*[20], using dialysed enzyme extracts of this *Pseudomonas*, showed that the initial product formed from protocatechuate was an acid possessing reactive carbonyl groups, and with an absorption spectrum shown in Fig. 3*a*. Ribbons and Evans[42] followed this reaction in a respirometer, and as soon as it reached completion, extracted the keto-acid into ethyl acetate. It was re-extracted into dilute aqueous sodium hydroxide or carbonate, giving a characteristic yellow colour to the solution (reminiscent of keto-enol tautomerism), which persisted. When dilute ammonia, however, was used, the yellow colour initially produced vanished within a few minutes, and a new compound was formed having spectrographic and chromatographic properties identical with 2 : 4-lutidinic acid. It is clear, therefore, that in the original experiments of Dagley and Patel[19], this compound was formed by a non-enzymic reaction between the initial product of ring-fission and the ammonium ion, present accidentally in the cell-free extract, since ammonium sulphate had been used for its fractionation. These facts support the formula, α-hydroxy-γ-carboxymuconic semialdehyde (6-formyl-4-carboxy-2-oxohex-4-enoic acid) we assign to the intermediate. It gives an acidic *bis*-2 : 4-dinitrophenylhydrazone, m.p. 170–2° (decomp.) and is somewhat unstable on recrystallization (Found : C, 39·8, 43·5; H, 3·4, 3·8; N, 19·2, 20·6; $C_{19}H_{14}N_8O_{13}$ requires C, 41·8; H, 2·6; N, 20·6 per cent).

When a non-dialysed cell-free preparation of this *Pseudomonas* is used to oxidize protocatechuate, α-hydroxy-γ-carboxymuconic semialdehyde is further metabolized to give a compound containing an additional carboxyl group, but the carbonyl groups have now disappeared. This second acid has a characteristic absorption maximum at 284 mμ and forms a *p*-bromophenacyl derivative (tri-ester) m.p. 101–2° ; the fact that it appears without any further gaseous exchange supports the view that an internal oxidation-reduction is involved, to give 2-hydroxy-4-carboxy-2 : 3-dihydromuconic acid (5-hydroxy-3-carboxyhex-2-enedioic acid). Concentrated, dialysed enzyme extracts degrade protocatechuate further, with the production of pyruvate as one end-product. In the presence of sodium arsenite (5×10^{-3} *M*) and/or pyridine-3-sulphonic acid (2×10^{-3} *M*), whole cells oxidize protocatechuate with substantial accumulation of pyruvate and oxaloacetate. The suggested sequence of reactions of the new pathway are depicted in Fig. 4. The fission proposed for 2-hydroxy-4-carboxy-2 : 3-dihydromuconic acid has not been demonstrated for cell-free systems; this investigation is in progress.

The two different ring-cleavage mechanisms of protocatechuic acid may be followed stoicheiometrically in an automatic titrator, as shown in Fig. 5*a* and *b* with the following results :

Dialysed, cell-free preparations from organisms employing the 3 : 4-split produce from protocatechuate two equivalents of acid, with the formation of *cis-cis*-β-carboxymuconate ; undialysed extracts produce approximately 1·3 equivalents with stoicheiometric formation of β-oxoadipate (one carboxyl group having been lost through decarboxylation).

Dialysed, cell-free preparations from microbes performing the 4 : 5-split acting on protocatechuate, give rise to one equivalent of acid with the formation of α-hydroxy-γ-carboxymuconic semialdehyde ($\lambda_{max.}$ 293 mμ (acid); 410 mμ (alkali)); undialysed extracts metabolize this keto-acid further, with the appearance of a second acid equivalent, corresponding to 2-hydroxy-4-carboxy-2 : 3-dihydromuconate ($\lambda_{max.}$ 284 mμ).

(2) *Cleavage of catechol by a new enzyme—'catechol 2 : 3-oxygenase'* : The degradation of catechol by two strains of Gram-negative bacteria has been investigated by Dagley and Stopher[21]. One was supplied by Dr. Eileen Pankhurst, London Research Station of the Gas Council, and readily grows with *m*-cresol as sole carbon source ; the other was isolated from garden soil, and utilizes *o*-cresol. Cell-free extracts from either organism oxidized 1 mole of catechol with the uptake of 1 mole of oxygen, but no carbon dioxide was evolved. During the reaction a transient yellow colour was produced, shown to be due to an

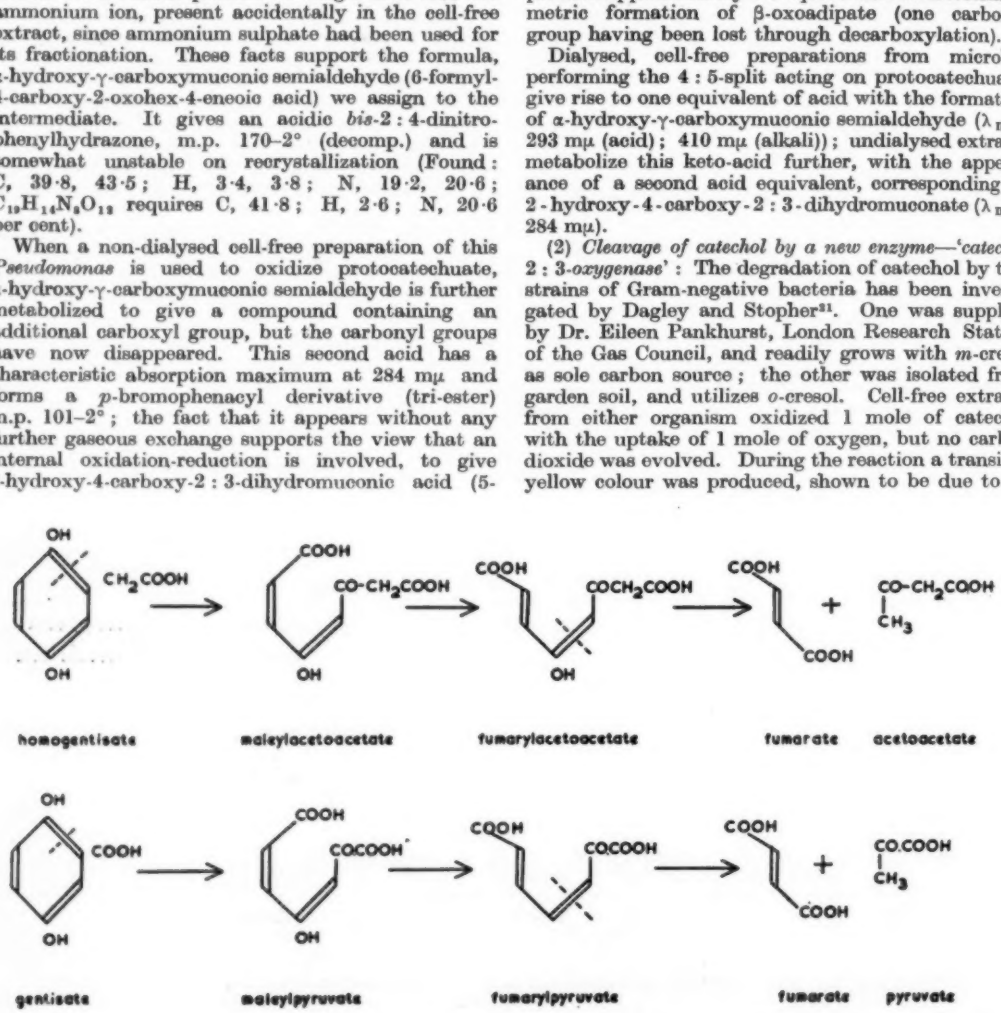

Fig. 2. Oxidative fission of *p*-dihydroxyphenols by micro-organisms and mammals

acidic intermediate; it gave an immediate bright orange colour with *o*-dianisidine (aldehyde test), and formed a *bis*-2 : 4-dinitrophenylhydrazone derivative. The keto-acid absorbed strongly at 373 mμ in neutral or alkaline solution; in acid this absorption and the yellow colour were abolished, and another peak appeared at 317 mμ (Fig. 3*b*). Clearly, this intermediate has similar properties to α-hydroxy-γ-carboxymuconic semialdehyde, although it differs in being much less reactive towards ammonia, since α-picolinic acid is formed only after standing with ammonium hydroxide for two days at room temperature[20]. Fortunately, it proved possible to isolate the intermediate itself. Stopher observed that when cell-free extracts of the *o*-cresol-utilizing *Pseudomonad* were maintained at 53° for 10 min., the enzymes that degraded the intermediate were destroyed, but not those involved in its production from catechol. By use of heat-treated extracts, a small quantity of an orange-coloured solid was isolated, which on analysis gave: C, 50·9; H, 4·3. α-Hydroxymuconic semialdehyde (6-formyl-2-oxohex-4-enoic acid), $C_6H_6O_4$ requires C, 50·8; H, 4·2 per cent. When reduced with platinum in ethanol, reactions for carbonyl groups were no longer given, and 3 moles of hydrogen were taken up per mole of substance, as required for reduction of two double bonds and one aldehyde group.

Untreated extracts gave pyruvate as one end-product of catechol oxidation, and the reaction could be followed in a spectrophotometer by measurements of absorption at 275 mμ ($\lambda_{max.}$ for catechol) and at 430 mμ where the intermediate absorbs (though not maximally) but catechol does not. During the course of this experiment the intermediate was rapidly formed and then degraded; *cis-cis*-muconic acid, present in a control vessel, was not attacked by the extract (Fig. 6). A heat-treated extract which decomposed catechol also attacked 4-methylcatechol to form an intermediate with an absorption spectrum similar to that from catechol ($\lambda_{max.} = 378$ mμ) but differing in some details. Various other phenolic compounds were not attacked.

For certain strains, notably the *Pseudomonas* of Dagley and Patel[19], ferrous ions can readily be shown to be a requirement for the enzymes catalysing the new mode of ring-fission but in other cases, the iron, if it should prove to be essential, is very strongly bound to the protein and cannot be removed by dialysis.

The product of ring-fission of catechol (α-hydroxymuconic semialdehyde) has already been encountered in work with mammalian systems. Wiss[22], and Mehler[23], have shown that an enzyme from liver oxidizes 3-hydroxyanthranilic acid to a derivative of muconic semialdehyde (Fig. 7). On standing in acid, carbon dioxide and ammonia are lost to form a compound, the spectrum of which agrees with that shown in Fig. 3*b*, and is similarly affected by changes in *p*H (ref. 23). Dr. Mehler kindly supplied us with a small amount of this compound; although not pure, material present which absorbed strongly at 375 mμ was rapidly decomposed by our cell-free extracts.

One product of the action of crude bacterial extracts on α-hydroxymuconic semialdehyde has been identified as pyruvate (Fig. 7), but details of the degradation are not yet known. By analogy with the reactions of Fig. 4 it might be suggested that the semialdehyde is first converted into 2-hydroxy-2 : 3-dihydromuconate, although in this case extracts have not been obtained which accumulate this compound. It is conceivable that 2-hydroxy-2 : 3-dihydromuconate would then undergo a hydrolytic split between carbon atoms 3 and 4 to produce malonic semialdehyde and lactate; a coupled oxidation-reduction would then produce pyruvate and β-hydroxypropionate.

Proctor and Scher[18] have recently investigated the metabolism of benzoate by a *Rhodopseudomonas* strain, and found that the pathway of degradation by this organism is different from those described to date, but consistent with the following sequence: benzoate → protocatechuate → catechol → an unidentified α-oxo acid which appears to be similar to α-hydroxymuconic semialdehyde. Extracts of this organism degraded catechol to an α-oxo acid only when hydrogen peroxide was added; oxygen would not be substituted. This system appears to differ therefore

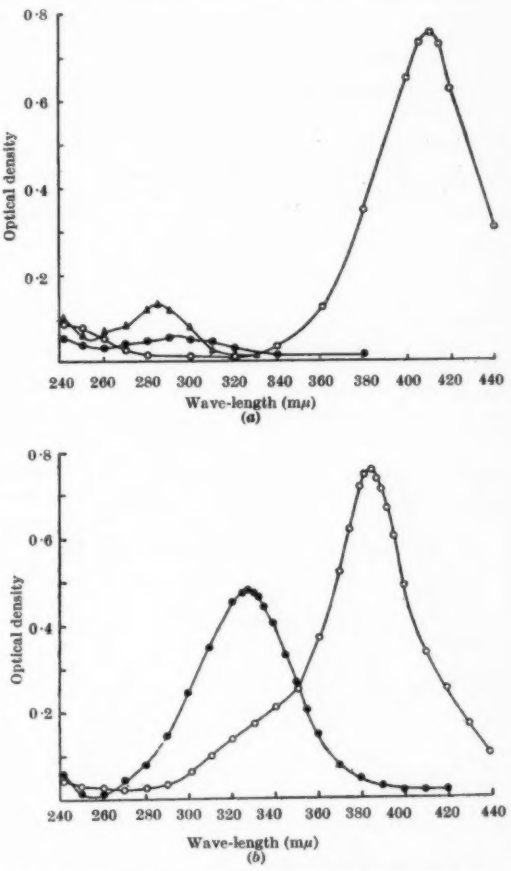

Fig. 3. Absorption spectra of the new ring fission products. *a*, Initial ring fission product (α-hydroxy-γ-carboxymuconic semialdehyde) of protocatechuate oxidation by protocatechuic acid 4 : 5-oxygenase. In acid (—●—), in alkali (—○—); molar extinction coefficient at $\lambda_{max.}$ = 410 mμ is 29,000. Also of the further metabolite, 2-hydroxy-4-carboxy-2 : 3-dihydromuconate (—△—): molar extinction coefficient at $\lambda_{max.}$ = 284 mμ is 5,000. Molar extinction coefficients are calculated on the basis of protocatechuate oxidized

b, Initial ring fission product (α-hydroxymuconic semialdehyde) of catechol oxidation by catechol 2 : 3-oxygenase. In acid (—●—), in alkali (—○—); molar extinction coefficient at $\lambda_{max.}$ = 373 mμ (*p*H 7·3) is 29,700, calculated for a solution of the purified compound containing 2·7 μgm./ml.

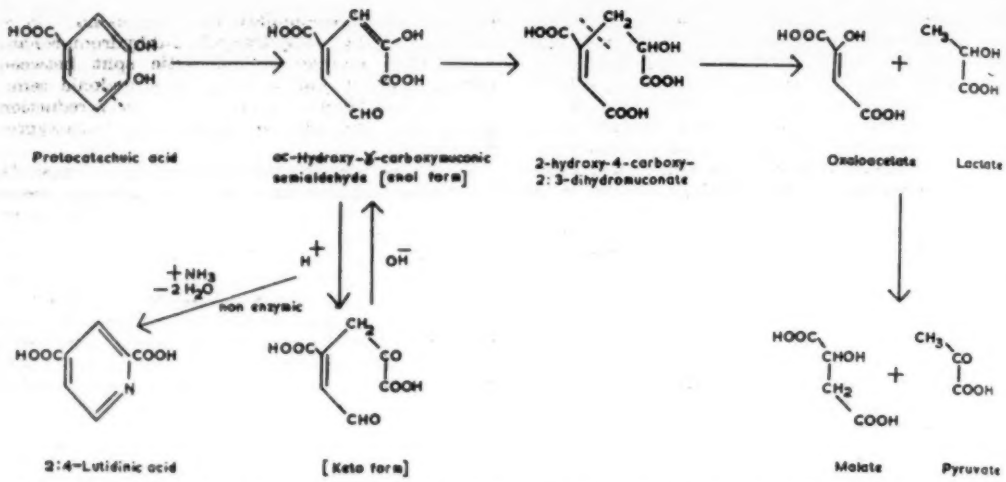

Fig. 4. Suggested new pathway of protocatechuate oxidation by micro-organisms which possess the protocatechuic acid 4 : 5-oxygenase system

from both pyrocatechase and catechol 2 : 3-oxygenase, and may well be yet another ring-fission enzyme. Their observations, published in abstract form, are complicated, since whole cells in the presence of oxygen or hydrogen peroxide produced a β-oxo acid (identity not disclosed) from catechol.

(3) *Variations in the subsequent pathway of cis-cis-β-carboxymuconic acid:* Several microbial species metabolize protocatechuate through *cis-cis*-β-carboxymuconate to the common intermediate β-oxoadipate; it now transpires that the intervening steps are not identical in all cases. Gross, Gafford and Tatum[8], using 2 : 6-$^{14}C_2$-labelled protocatechuic acid, showed that the carbonyl-C of β-oxoadipate produced by a *Neurospora crassa* enzyme system originated solely from C-6 of protocatechuate, whereas using a *Pseudomonas fluorescens* system (strain *A*, 3·12, Stanier) it was randomly derived from C-1 and C-6 of this substrate. This *Pseudomonas* route diverges from the *Neurospora* pathway (shown in Fig. 1*b*) after the *cis-cis*-β-carboxymuconate stage, since β-carboxymuconolactone was not attacked; *cis-cis*-muconate and muconolactone were not degraded by either of these enzyme systems. The tracer results for this *Pseudomonas* pathway imply the formation of a symmetrical precursor to β-oxoadipate, which

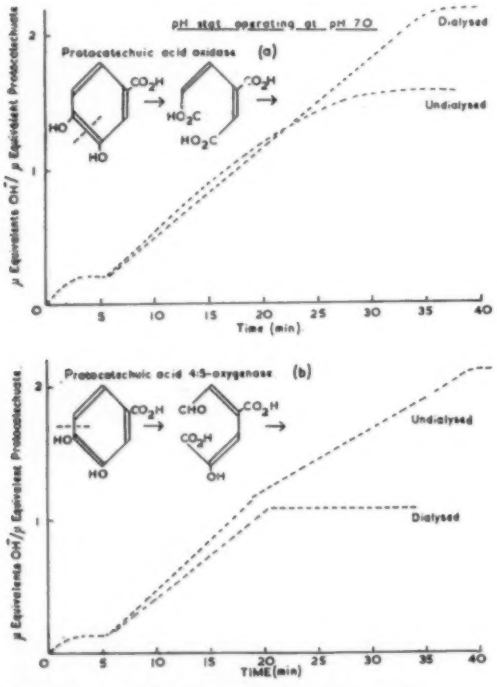

Fig. 5. A comparison of acid production during ring fission and further metabolism of protocatechuate by: *a*, protocatechuic acid oxidase; and *b*, protocatechuic acid 4 : 5-oxygenase

The reaction cuvette contained enzyme solution (sonic extracts in distilled water, 1 ml.) as indicated, and distilled water (4 ml.), brought to *p*H 7. Temperature, 30°. Reactions were initiated by addition of protocatechuate (10 μmoles in 1 ml. water, previously neutralized to *p*H 7). The micro-burette contained 0·1 *N* sodium hydroxide, and automatic additions during reaction were recorded graphically (Radiometer, Ltd., Copenhagen)

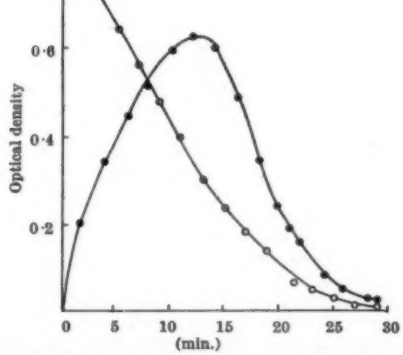

Fig. 6. Spectrophotometric assay of catechol oxidation by the catechol 2 : 3-oxygenase system. Optical density due to catechol at 275 mμ (—○—); optical density due to α-hydroxymuconic semialdehyde at 430 mμ (—●—)

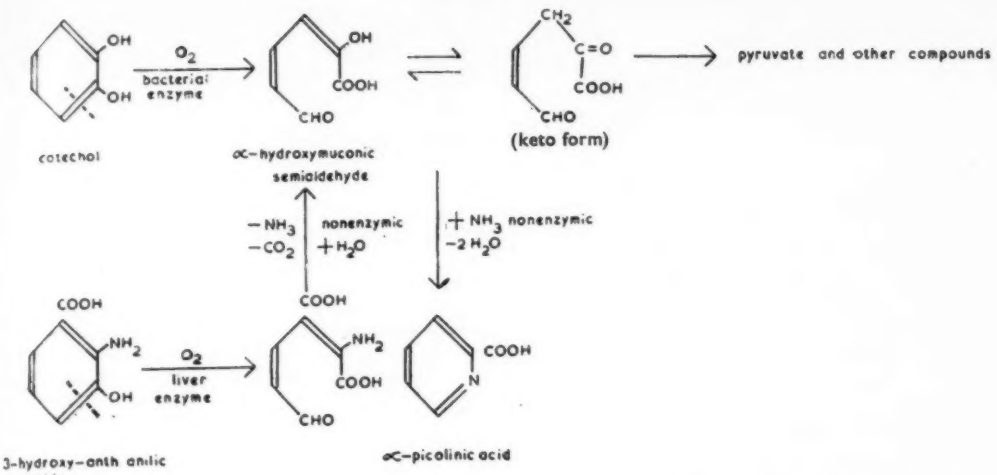

Fig. 7. Suggested new pathway of catechol oxidation by micro-organisms possessing the catechol 2 : 3-oxygenase system. The formation of α-hydroxymuconic semialdehyde from catechol, and its relation to the ring fission product of 3-hydroxyanthranilate by the liver enzyme, is indicated

Elsden and Peel[24] suggested might be the dilactone of *cis-cis*-muconic acid (butanolido-βγ-γ′β′-butanolide). Cain, Ribbons and Evans[25] found that this dilactone gave rise to β-oxoadipate with extracts from another *Pseudomonas* strain and *Vibrio* 0/1 induced to protocatechuate. At slightly acid, neutral and alkaline *p*H, however, the dilactone itself underwent a non-enzymic rearrangement to muconolactone. Moreover, muconolactone also produced β-oxoadipate with these enzyme systems. The spontaneous hydrolysis of the dilactone to muconolactone followed by enzymic attack on this product is responsible for apparent attack on the dilactone itself; this dilactone cannot therefore be an intermediate in these particular systems, but ββ′-dihydroxyadipate and carboxydilactone are further possibilities to be explored. Cain[15] has shown that muconolactone, which he rigidly identified, accumulated when heat-treated extracts of *Vibrio* 0/1 grown on *p*-hydroxybenzoate oxidized protocatechuate with consumption of 1 mole of oxygen and the evolution of 1 mole of carbon dioxide per mole of substrate. From these results it is probable that muconolactone is an intermediate in the conversion of *cis-cis*-β-carboxymuconate to β-oxoadipate by *Vibrio* 0/1 and by some, but not all, *Pseudomonas* strains (Fig. 8).

Yet another route from *cis-cis*-β-carboxymuconate to β-oxoadipate is taken by *Nocardia erythropolis* according to the work of Cain and Cartwright[16]. A lactone-like intermediate is produced having $\lambda_{max.}$ at 215–218 mμ; it differs from any known lactone of these series, and awaits identification.

(4) *Diversity of pathways in the oxidative metabolism of aromatic compounds prior to ring fission.* Hayaishi and Stanier[26] showed that there were two distinct pathways of tryptophan metabolism among the *Pseudomonads*. Most organisms oxidize this amino-acid through kynurenine, anthranilate and catechol; some strains diverge from this sequence at the kynurenine stage, producing kynurenic acid, the subsequent fate of which has been partially clarified[27].

Microbial dissimilation of phenylacetate shows an analogous dichotomy, as demonstrated by Kunita; one strain of *Pseudomonas fluorescens* produces homoprotocatechuate[28] from this substrate, while another appears to take the homogentisate route[29].

The pathways employed for the utilization of the benzoates among microbial species and strains prior to ring-fission, discovered to date, are illustrated with references in Fig. 9; a more remarkable diversity than was at one time supposed is clearly evident.

The enzymic transformations involved in these reactions are hydroxylations or perhydroxylations (followed by dehydrogenation), and decarboxylations or oxidative decarboxylations. The particular route taken by a micro-organism capable of utilizing an aromatic compound will presumably depend on its genetic potential, coupled with the influence of

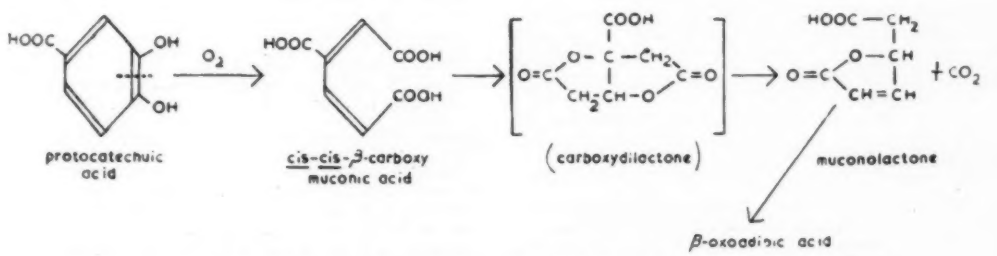

Fig. 8. Pathway of dissimilation of protocatechuate by *Vibrio* 0/1. The carboxydilactone step is hypothetical

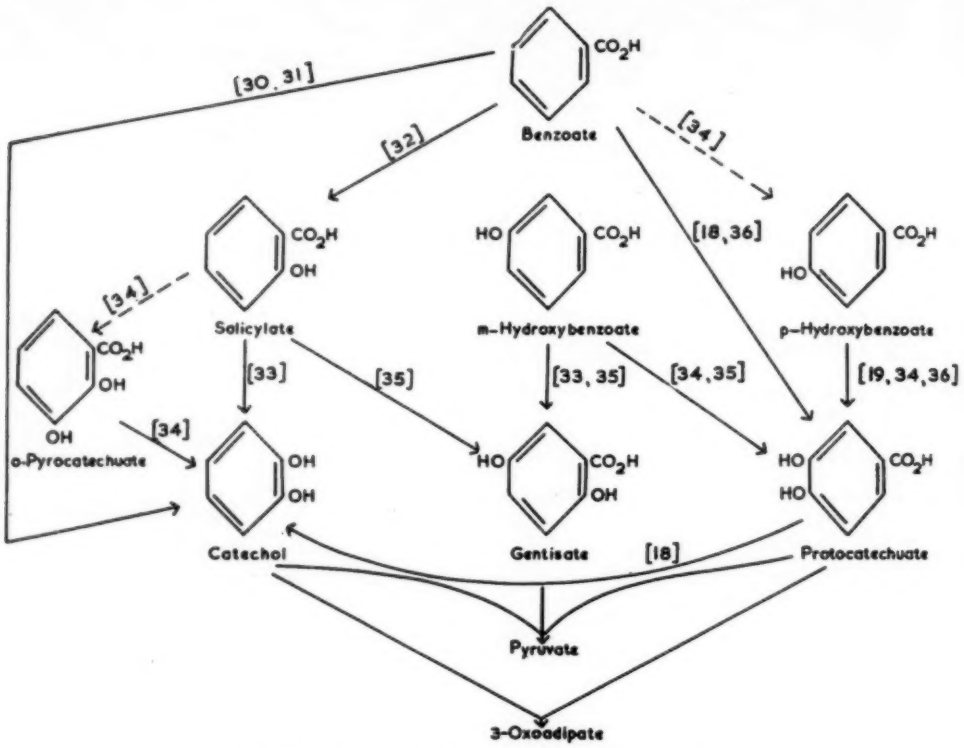

Fig. 9. Diversity in metabolism of the benzoates by micro-organisms

environment, for the syntheses of the relevant enzymes.

Micro-organisms which degrade polycyclic aromatic compounds appear to employ a stepwise, end-ring attack (for example, naphthalene, anthracene, phenanthrene and their derivatives)[38–40]. The discovery of this new method of ring cleavage now makes it probable that there exist several alternative routes for the metabolism of condensed ring systems. Similarly, the metabolism of herbicides of aromatic nature by soil microbes[40,41] may well show diversity due to species and/or strains.

Although we have no statistics as to the relative prevalence of the various modes of ring fission, bacteria which employ all of them abound in the soil.

We wish to thank the Department of Scientific and Industrial Research and the Agricultural Research Council for their financial support.

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LETTERS TO THE EDITORS

GEOPHYSICS

Formation of the Sporadic *E* Layer in the Temperate Zones

In a recent communication Heisler and Whitehead[1] noted that the fraction of time the critical frequency of the sporadic E layer (f_0E_s) exceeded 5 Mc./s. depended on the horizontal component of the Earth's magnetic field, increasing as the horizontal component increased. This relationship is consistent with sporadic E formation arising from a vertical gradient of horizontal movement of the neutral air. In a region where the gyro-frequency of the ions, ω_{Hi}, is less than their collision frequency, ν_i, it is possible to show that, if there is no vertical electric current and the net horizontal current is zero (a horizontal current at one height being cancelled by a reverse current at some other height), the vertical velocity of ions and electrons is given approximately by :

$$V_z \sim \frac{-\omega_{Hi} \cos\theta}{\nu_i} \cdot \Delta U_y$$

where θ is the dip angle and ΔU_y is the departure of the horizontal wind velocity at right angles to the Earth's magnetic field from the mean value in the region. A thin layer of ionization will form where V_z is zero but where the vertical gradient of V_z is large. The probability of such formation depends on $\omega_{Hi} \cos\theta$, which is proportional to the horizontal component of the Earth's magnetic field.

Thus the global distribution of E_s in the temperate zones may be explained.

A more detailed analysis shows that at the height at which an E_s layer is already formed, ΔU_y, and hence V_z, remain nearly zero, independent of the horizontal winds. Thus the E_s layer continues to form at the same height. This explains the persistence of the E_s layer even when the horizontal winds leading to its original formation might be expected to have changed.

The detailed analysis will be presented elsewhere.

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Telluric Current Micropulsations on Arctic Drifting Station Charlie

Telluric current recording equipment was operated on Arctic Drifting Station Charlie during the epoch October 15–December 31, 1959. The station was located on a 4-mile by 7-mile ice floe of quite uniform thickness, the thickness averaging perhaps 10 ft. The electrodes consisted of 8-ft. by 2-in. lead strips prepared from electric cable sheathing. Electrode pairs were cut from continuous lengths of sheathing to assure identity of material in the two electrodes. No difficulty was experienced with contact potentials. The d.c. voltage never exceeded a few millivolts and was very steady. The electrodes were suspended in the sea just below the ice at a spacing of 1·082 km. The relatively long spacing was necessary because of the low mV./km. values experienced over the highly conducting sea water.

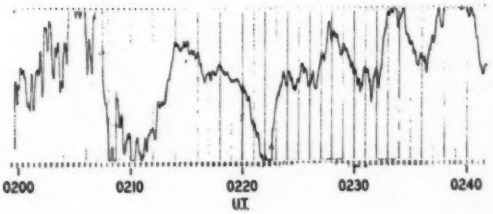
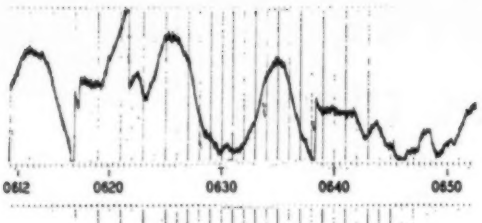


Fig. 1. Telluric current, Arctic Drifting Station Charlie, December 11, 1959

On December 11, 1959, micropulsations of less than 1-sec. period were recorded for an interval of more than 2 hr. On this date the floe was located at 75° 57′ N. and 171° 01′ W., which corresponds to geomagnetic co-ordinates of 70° 55′ N. and 222° 33′ E. During the afternoon of this day (0200–0240 U.T.) a very sensitive recorder, 2 mV. full scale on the 1·082 km. spaced electrodes, was operated to search for micropulsations. Even though this was a very quiet period, based on normal recording, it was necessary to return the pen to scale several times by inserting a voltage in the electrode lines. This trace is presented as Fig. 1. A slight widening of the trace is evident, but the effect is so small as to be inconclusive as an indication of the occurrence of micropulsations.

On returning to the laboratory in the evening a quiet auroral arc was observed. The arc extended to the horizons and passed through the zenith. The normal sensitivity telluric current recorders were essentially quiet, the normal condition during such an auroral display. The high-sensitivity recorder was reconnected to the electrodes and the trace of Fig. 2 recorded. It is impossible even on the original trace to discern the individual fluctuations. However, the upward motions of the pen were counted by visual observation for six 1-min. intervals, extended over an hour of the activity. These counts gave 70, 70, 70, 67, 68, and 70 cycles per minute. It was also noted that the rate of fluctuation tended to be very uniform throughout the activity. The amplitude of the pulsations ranged from 0·05 to 0·10 mV./km. The circumstances of the experiment assure beyond any reasonable doubt that these were natural micropulsations and not variations introduced by the instrumentation.

No previous record of telluric current micropulsations of this short period has come to my attention.



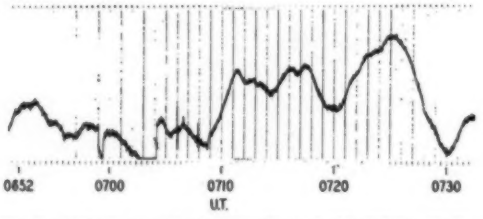


Fig. 2. Telluric current micropulsations, Arctic Drifting Station Charlie, December 11, 1959

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PHYSICS

Shock Excitation of Solid Aromatic Hydrocarbons

Shock excitation of a wide range of powdered inorganic solids (mainly metallic oxides) by helium-driven shock-waves in argon[1] has not only provided a powerful means of producing relatively clean spectra of astrophysically important diatomic molecules[2], but also has given some insight into the physical processes which take place when the powder particles undergo severe thermal transients. From the general observation that molecular spectra alone are excited (in the hot stagnant region behind the reflected shock wave) at low temperatures (~2,000° K.), mixed molecular and atomic spectra are excited at intermediate temperatures (~5,000° K.) and entirely atomic spectra are excited at high temperatures (~10,000° K.), and from other experimental considerations (refs. 1*a* and 1*c*), it is thought that a predominantly atomic vapour is evaporated from the solid inorganic surfaces, which, if the temperature is low enough, partially or completely recombines to form diatomic species. It is therefore a logical extension of this work to examine the variation with environmental temperature of the spectra from shock excitation of powdered organic materials, and in this communication experiments with benzene, naphthalene, and anthracene are discussed.

Greene[3] has examined the spectra from shock excitation of the aliphatic vapours: (*a*) XCN (X = Br, Cl); (*b*) CX_3Y (X = H, F; Y = Br, I) and reports the presence of CN violet bands and C_2 Swan bands in the case of (*a*), and a carbon particle continuum, C_2 Swan bands, and a CH band in the case of (*b*). Fairbairn and Gaydon[4] have reported spectra from the shock excitation of a much wider variety of aliphatic vapours, and find that the carbon particle continuum and the C_2 Swan bands predominate while OH and CN sometimes appear. They find no trace of CH even for molecules containing C and H. Johnson and Campbell[5] report CH and C_2 bands in absorption through shock-heated benzene vapour.

In our experiments a few grams of each powdered solid to be studied were placed at the end window of a small shock-tube (except in the case of benzene, which was frozen on to the inner wall of the shock tube near the window by means of an external dry ice jacket). A series of spectra were photographed on a Hilger *f*/4 spectrograph over as large a range of calculated stagnant-region temperatures as possible. These temperatures are undoubtedly inaccurate as they take no account of the contamination of the argon with organic vapours. However, Parkinson and Nicholls (refs. 1*d*, 6) have shown that (in shock-excitation of inorganic solids) the rotational temperature of the CN molecule is only about 10–15 per cent lower than the calculated gas kinetic temperature, and in good agreement with the temperature inferred from the measured shock-speed. The shock tube was cleaned and recharged with organic material between each exposure.

In each case the main contributions to the spectra were the (0;0,1,2) (1;0,1,2,3) (2·0,1,2,3,4) (3;1,2,3,4,5) (4;2,3,5,6) (5;6) C_2 Swan bands, the (0;0,1) (1;1,2)

Table 1. Spectra from Shock Excitation of Solid Benzene, Naphthalene and Anthracene

Temperature range used	Benzene 4,900–8,440° K.			Naphthalene 3,890–13,000° K.			Anthracene 3,630–12,020° K.		
	Temp. of appearance	Temp. of maximum intensity	Comments	Temp. of appearance	Temp. of maximum intensity	Comments	Temp. of appearance	Temp. of maximum intensity	Comments
Atomic impurities (Na, Ca)	4,900°	6,990°	Present at lowest temp. Not present at 8,440°	3,890°	6,870°	Not present at 13,000°	—	—	—
C-particle continuum	—	—	Benzene not in powder form	3,890°	4,800°	Reaches low value at 6,900°, remains until 13,000°	3,630°	5,950°	Reaches low value at 6,350° and remains constant to 12,020°
C_2 Swan bands	4,900°	6,990°	Weak at 8,440°	4,800°	10,250° ?	Constant to 13,000°	5,950°	6,350°	Weak at 12,020°
CN violet bands	4,900° (weak)	6,990°	Not present at 8,440°	6,080°	?	Gradual increase to 13,000°	4,970°	5,950°	Absent above 8,600°
CH λ4315 Å. band	6,990°	?	Present at 8,440°	6,870°	?	Gradual increase to 13,000°	5,950°	6,350°	Absent above 8,600°

(2;2,3) (3;3,4) (4,5) (5,6) CN violet bands (nitrogen was an impurity in the argon), the (0,0) CH band (4315 Å.), and (except in the case of benzene) a carbon particle continuum the intensity and spectral range of which were extremely sensitive to temperature. The circumstances in which the various spectra appear are summarized in Table 1. The lack of carbon-particle continuum in the case of benzene is probably due to the fact that it is not powdered but a solid coating on the wall of the tube. In the case of the other two compounds, the powdered configuration and consequent large surface area gave greater chance for pyrolysis in the hot gas. Abundant solid carbon particles were present in the shock tube after each experiment.

There is a striking contrast between these entirely molecular spectra (except for a few atomic impurity lines due to sodium and calcium) over the entire temperature-range, and the smooth transition with increasing temperature from molecular to atomic spectra observed in the case of the inorganic compounds. It is therefore doubtful whether the same mechanism is operative in both cases. It seems more probable (in view of the much lower volatilization temperatures of the organic compounds) that the shock treatment causes evaporation of a molecular vapour which is pyrolysed to carbon at lower temperatures (possibly by Porter's acetylene mechanism[7] confirmed by Greene, Taylor and Patterson[8] for a number of simple hydrocarbon vapours). At higher temperatures reactions between the carbon vapour and other gases (nitrogen and hydrogen), present in the tube following pyrolysis, qualitatively explain the CH and CN spectra. Spectra of atomic carbon are not observed in the spectral range of our instrument because of the inaccessibility to thermal excitation (at the temperatures used) of the upper states of allowed transitions in C.

This work has been supported in part by contracts with the Department of Defence Production of Canada and the U.S. Air Force Cambridge Research Center, and in part by research grants from the Defence Research Board of Canada, the National Research Council of Canada and the Ontario Research Foundation. One of us (M. D. W.) gratefully acknowledges the award of scholarships by the Ontario Research Foundation.

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The Critical Phenomena between Solids and Fluids

In the phase diagrams of simple pure substances, the unknown fundamental problem is only the upper limits of melting points. Is there any critical phenomena between solids and fluids? (Generally at their melting points solids change to gases without going through the liquid stage in the high-temperature and high-pressure range.)

Experimental research on this problem was begun by the late Sir Francis Simon and his school thirty years ago. Up to the present, investigations have been carried out up to 7,500 atm. for helium[1] and 10,000 atm. for nitrogen and argon[2], but there is no evidence of the existence of critical points. Therefore Prof. P. W. Bridgman[3] and Prof. C. Domb[4] and the others have been inclined to doubt the existence of this point. Profs. L. Landau and E. Lifschitz[5] have also stated definitely there is no intermediate state between crystal and liquidus, for these states have distinct characteristics of internal symmetry.

As this problem is of the utmost importance for understanding the structure of liquids too, I wish to discuss the problem in connexion with my structural model of liquids[6].

Recently, Prof. J. D. Bernal[7] reached the important conclusion that "there is an absolute impossibility of forming a homogeneous assembly of points of volume intermediate between those of long-range order and closest packed disorder". Therefore, by the geometrical model with its static characteristics, the liquidus (disordered) state cannot truly change to the solid (ordered) state continuously. Meanwhile[6] I have presented a simple structural model of liquids with dynamical characteristics, which is consistent with Bernal's model. From this model, the structure of liquids near their melting points is approximated by a quasi-face-centred cubic lattice in which there are many vacant sites occupying about 10 per cent of the structure, but these empty spaces are distributed through all the interstices, causing the disappearance of the long-range order of lattice. Therefore, if the concentration of vacant sites is increased up to several per cent in crystals at sufficiently high temperatures and pressures, the regular lattices of the crystals will be gradually distorted under these conditions, resulting in irregular liquid structures.

The free-energy change ΔG in going from the regular perfect crystals to this intermediate state at constant temperature and pressure is:

$$\Delta G = \Delta E + p.\Delta V - T.\Delta S \qquad (1)$$

where ΔE, ΔV and ΔS are the changes of internal energy, volume and entropy respectively. Generally, the first and second terms of the right-hand side of equation (1) are positive, and the third is negative; and generally $\Delta G > 0$, corresponding only to the low concentration of vacant sites in crystals at melting points under ordinary pressures. But, in highly compressed condensed systems, if the equilibrium positions of the bottoms of molecular potential wells are higher than zero, which corresponds to the potential energy of free gas molecules (see Fig. 1), ΔE will become negative, and in the higher temperature range $-T.\Delta S$ will also take large negative values, with the result that $\Delta G < 0$. Therefore in these highly compressed and high-temperature conditions the vacant sites will be produced spontaneously in crystals.

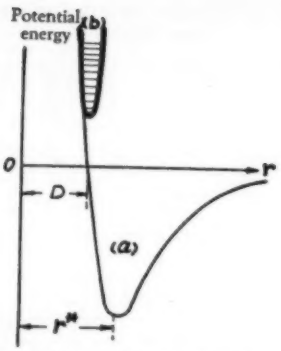

Fig. 1. Intermolecular potential curve (*a*) and a potential well of a molecule in the condensed phase (*b*)

In general, the following formulæ representing the intermolecular potentials φ is applicable to monatomic van der Waals' and metallic liquids, including spherico-symmetrical molecular liquids[8]:

$$\varphi = -ar^{-m} + br^{-n}, \quad m < n$$

and

$$\frac{D}{r^*} = \left(\frac{m}{n}\right)^{1/(n-m)}$$

where r^* is the distance apart of molecules at the minimum potential and D is the distance at zero energy-level (see Fig. 1).

Table 1. Estimated Critical Points between Solids and Liquids

	P_c (atm.)	T_c (° K.)	V_c (c.c./mol.)	P_cV_c/RT_c	m	n
He	11–14 × 10³	65–75	6·5–6·0	14	6	12
N_2	22·8 × 10³*	302*	20	18·5	6	12
CCl_4	17·4 × 10³*	631*	60	20·2	6	12
K	2–3 × 10³	700–1,000	15–12	45	2	4
Fe	3–4 × 10⁶	3,000–4,000	3·9–3·5	40	4	7

* Values estimated by Ebert in ref. 8.

In 1954 the late Prof. L. Ebert[8] estimated the critical temperatures T_c and pressures P_c of nitrogen, carbon tetrachloride and other gases by ingenious extrapolations from Bridgman's experimental data (see Table 1). From his values I have estimated the critical molar volumes V_c of nitrogen and carbon tetrachloride using Bridgman's data[9] (see Table 1), and found that these values are a little smaller than the volume calculated from D and thus support my views. In an infinitesimal change of the intermediate state of critical regions, $\delta G = 0$ and (1) becomes:

$$T_c\,(\partial S/\partial V)_{P_cT_c} = P_c + (\partial E/\partial V)_{P_cT_c} \qquad (2)$$

and we can explain approximately and verify this equation for nitrogen by using the data of Table 1 and my model.

Therefore, if φ is known, V_c can be calculated approximately. Next, by the extrapolation of experimental compressibilities and fusion curves, we can estimate P_c and T_c in an effort to make the above values consistent. This procedure is made easier by using the hypothesis that the non-dimensional quantity P_cV_c/RT_c will not vary as widely in several substances. This hypothesis is supported by the fact that at the critical points between gases and liquids, it is 0·29 for the spherico-symmetrical van der Waals' gases, which have values of $n = 12$ and $m = 6$, and 0·46 ~ 0·77 for mercury[10], which has lower values of n and m than the above (see Table 2).

My estimated critical values are given in Table 1. For helium I used the value of φ from Johnston and Grilly[11], the fusion curve of Holland, Huggill and Jones[1] and the molar volume of Dugdale and Simon[12]; for potassium, φ ($m = 2$, $n = 4$) was from Birch[13] and Gilvarry[14] and the data of Bridgman[3]; for iron φ ($m = 4$, $n = 7$) was from Fürth[15], the density estimated by Birch[16] and Valle[17], with experimental compressibility from Walsh, Rice, McQueen and Yarger[18], and the fusion equation from Strong[19].

Table 2. Experimental Critical Points between Gases and Liquids

	P_c (atm.)	T_c (° K.)	V_c (c.c./mol.)	P_cV_c/RT_c	m	n
He	2·26	5·3	57·8	0·30	6	12
N_2	33·5	126	90·2	0·29	6	12
Hg	1,640[10]	1,730[10]	67–40[10]	0·46–0·77	6 ?[11]	8 ?[11]

My values for helium are about twice as large as those of the previous experimental region. The values for potassium and iron only have a meaning concerning the order of magnitudes owing to the low reliabilities of φ and other data, and owing to their body-centred cubic structure, which is not the close-packed structure. But it is interesting that the values for iron are near those for the Earth's core. Moreover, on the surface of the Earth's core, putting $P = 1{\cdot}4 \times 10^6$ atm., $V = 5{\cdot}74$ c.c./mol. for iron and $T = 2{,}600°$ K., which is the melting point of iron calculated from Strong's equation[19], we obtain a non-dimensional quantity $PV/RT = 37{\cdot}7$, very near that of the estimated critical point of iron. Hence it seems likely that there is an internal relation between the critical phenomena and the fluid characteristics of the Earth's core.

In any event, it will be valuable to examine, by modern techniques, the critical limits of the melting points of nitrogen or helium at pressures about twice as high or more as those used in previous experiments.

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Velocity of Ultrasonic Waves in Aqueous Solutions of Zinc Halides

Zinc and cadmium belong to sub-group II of the Periodic System. Their compounds exhibit properties characteristic of the second group. Deviations from the normal thermodynamic behaviour have been reported in the case of halides of these metals[1].

Investigation by ultrasonic radiation[2,3] of aqueous solutions of cadmium halides showed interesting deviations from the normal behaviour of electrolytic solutions. Hence, it is expected that zinc halides would also exhibit similar anomalies.

Ultrasonic velocity as a function of concentration has been studied using a variable-path, single-crystal interferometer, working at 780 kc./s. Velocity measurements were made at 32 ± 0·5° C., and are accurate to ± 0·15 per cent.

Experimental results showing the variation of velocity with molar concentration are presented in Fig. 1. All the halides show exceptional behaviour. In the case of zinc chloride, the velocity increases with concentration up to 0·25 *M*, after which it decreases continuously, reaching a value of 1,503 m./s. at 1·5 *M*. Thereafter the velocity remains constant in the concentration-range studied (up to 2·5 *M*). Zinc bromide also exhibits a similar tendency, but to a lesser degree; up to 0·5 *M* the velocity remains practically constant and thereafter it decreases. Zinc iodide shows a continuous decrease with increasing concentration.

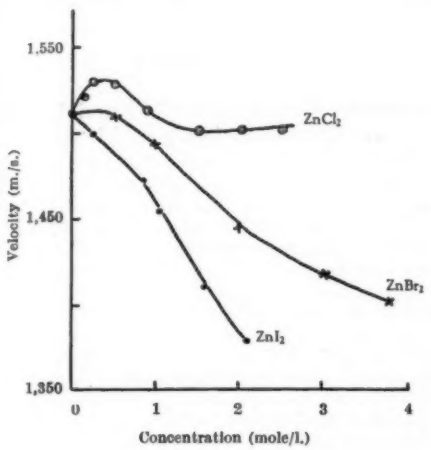

Fig. 1

Balachandran[3] has studied the ultrasonic behaviour of zinc iodide in the concentration-range 0–0·5 *M*. His results also indicate decrease of velocity of sound with increasing concentration.

Adiabatic compressibility decreases with increasing concentration in all three cases. At any molar concentration, the decrease in compressibility of water is a maximum in the case of iodide and a minimum in the case of chloride. It is to be noted that this order is the reverse of that found among cadmium halides[3].

Full details of this investigation will be published shortly. I thank Dr. J. Bhimasenachar for his encouragement and helpful discussions.

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The Unit of Neutron Flux

Having lectured for several years on the physics of nuclear reactors, we have often felt the need for a unit of neutron flux. It is comparatively easy to write "ns per cm.² per sec.", but much more clumsy to speak aloud the expression "neutrons per square centimetre per second" while lecturing.

We have adopted the name 'chad' as the unit of neutron flux in our lectures, in honour of Sir James Chadwick, and we would like to suggest that this name becomes the accepted unit, defined as follows:

1 chad ≡ 1 neutron per cm.² per sec.

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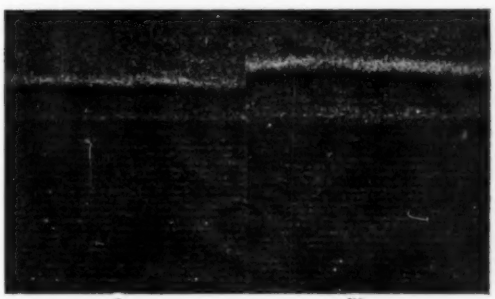
Electron Microscope Out-of-focus Image of the Edge of a Crystal Lattice

In 1957, Cowley and Moodie[1] pointed out, theoretically and by optical analogue experiments, that so-called Fourier images can be observed in out-of-focus images of a periodic object. According to them, Fourier images are interference fringe systems arising from the transmitted primary and several diffracted waves, and are a series of images in particular planes both in front of and behind the object and having the same geometrical form as the periodic object. In electron microscope images, passing through the focus, of copper–phthalocyanine, Komoda[2] observed that the periodic structure in the image appeared again after it had become diffuse. He concluded that the periodic structures appearing at a different focus are the Fourier images of the crystal lattice predicted by Cowley and Moodie. In the out-of-focus image of copper–phthalocyanine crystal taken by Komoda, we have detected a few extra lines outside an edge of the crystal (see Fig. 1). Such lines are not explicable by the theory of Cowley and Moodie. By using a light optical analogue, it has been shown that these lines are due to effects arising from the termination effect of the Fourier images and Fresnel diffraction of electron waves at the edge of the crystal.

Fig. 2 is a schematic diagram which demonstrates the Fourier images on the exit side of a grating for



(*a*) (*b*)

Fig. 1. Fringes of copper–phthalocyanine crystal for in-focus (*a*) and out-of-focus (*b*) image. By courtesy of Komoda. (× 433,000)

the case of $n = 0, \pm 1$ waves, where n is the order of reflexion. The spacing of the fringes is equal to that of the grating, and the intensity alternates as the distance from the grating becomes larger, with increase in order of the Fourier images, $N = 0, \pm 1/2, \pm 1, \pm 3/2, \ldots$ (negative sign indicates a virtual image). From Fig. 2 it can be seen that the number of fringes increases as the Fourier image of higher order is focused; for example, in the Fourier image of $N = 1/2$ one more fringe appears than in the image of $N = 0$. In the out-of-focus image of a crystal edge, Fresnel diffraction takes place at the edge both for beams which pass inside and outside of the crystal. Then the character of the fringes is slightly changed from that expected from Fig. 2. These effects can easily be demonstrated optically by using a plane grating and light.

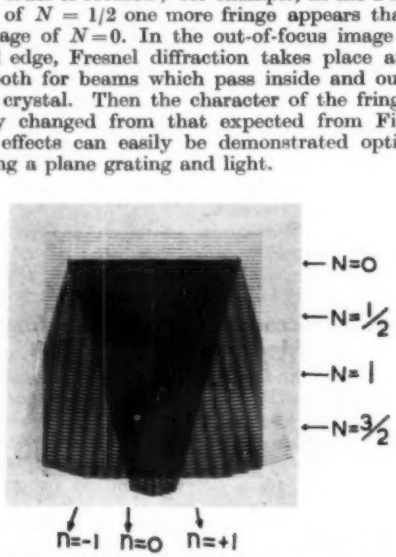
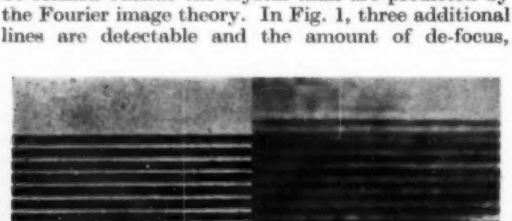


Fig. 2. Mode of interference of the electron waves passing through the lattice

In our demonstration, a plane grating of 66-μ spacing with an edge was irradiated by an almost parallel light beam of wave-length 4900 Å. This corresponds to a thin crystal of 6·6 Å. lattice spacing with an edge, which is irradiated by a 60-keV. electron beam. Figs. 3*a* and *b* are images of the plane grating obtained by focusing on the Fourier images $N = 0$ and $N = 1/2$ respectively. One more line is clearly formed outside the grating, and two more faint lines are also detectable. From this experiment, we can expect in the out-of-focus image of the crystal lattice many more extra lines would be formed outside the crystal than are predicted by the Fourier image theory. In Fig. 1, three additional lines are detectable and the amount of de-focus, which is estimated from the Fresnel fringe at the edge of the contaminating substance, is about 0·5μ, which corresponds to $N \doteq 1/2$. The number of extra lines outside the crystal agrees with that in the optical model.



(*a*) (*b*)

Fig. 3. (*a*) and (*b*) are the fringes in the Fourier images of $N = 0$ and $N = 1/2$ respectively. In (*b*) one strong and two faint fringes can be observed outside the grating

In a real crystal the intensity of beams reflected by the lattice planes is expected to vary according to the thickness and the inner potential of the crystal and to the amount of deviation from the Bragg angle, and a full analysis using the dynamical theory, taking these quantities into account, will be published shortly.

We thank Prof. K. Tanaka, Prof. K. Kobayashi and Dr. J. W. Menter for their helpful discussions.

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METALLURGY

Potential-Time Curves obtained during the Stress Cracking of Metals

We have investigated some of the electrochemistry of two 12 per cent chromium martensitic steels while undergoing cracking in acid media. Since it is known that these steels can be made to crack through hydrogen embrittlement as well as by stress corrosion, we were curious as to the cause of cracking in 1 per cent hydrochloric acid with 1 per cent dissolved selenium dioxide. Following the procedure of Brown[1], increasingly larger polarizing currents, both anodic and cathodic, were applied to stressed beam specimens to determine which reaction hastened the time to failure. From these results we concluded that the cracking in this medium was by hydrogen embrittlement. Further proof was obtained by substituting sulphuric acid for hydrochloric acid (to eliminate the possible stress corroding influence of the chloride ion) without any change in the pattern of results. Simultaneously with our tests on the effect of applied currents on time to fracture, potential-time curves were automatically recorded. The rather unusual changes in potential we observed are primarily the reason for this communication.

Potential-time curves have been plotted by several investigators studying the stress corrosion cracking of metals[2–4]. We are not aware of similar curves for hydrogen cracking. As a means of reference we have summarized the characteristics of potential-time curves obtained when austenitic stainless steels undergo stress corrosion cracking in magnesium chloride and mild steels stress-crack in boiling nitrate solutions. Certain of these features are also duplicated by aluminium and magnesium alloys in chloride environments.

(1) There is a gradual rise in potential in the noble direction immediately after the specimen is exposed to the cracking medium. The shape of this portion of the curve is independent of applied load.

(2) After a limiting potential is reached, breaks may occur in the potential-time curves when the

specimen is under load. These breaks are evidenced by very rapid drops in potential to a more active value and fairly rapid return to the 'normal' value.

(3) When the specimen finally fractures by stress corrosion, there is a very rapid drop to a more active potential.

(4) Application of cathodic current delays or possibly prevents failure. Anodic polarization may hasten time to failure.

The following outline describes the almost completely opposite effects found with the 12 per cent chromium steels: *A*, carbon, 0·129 per cent; manganese, 0·55 per cent; silicon, 0·40 per cent; phosphorus, 0·012 per cent; sulphur, 0·012 per cent; chromium, 12·15 per cent; nickel, 0·38 per cent; molybdenum, 0·02 per cent; copper, 0·11 per cent; *B*, carbon, 0·050 per cent; manganese, 0·63 per cent; silicon, 0·26 per cent; phosphorus, 0·012 per cent; sulphur, 0·017 per cent; chromium, 11·73 per cent; nickel, 1·76 per cent; molybdenum, 0·01 per cent; copper, 0·06 per cent; stressed to approximately 80 per cent of their yield strengths in a room temperature solution of 1 per cent selenium dioxide in 1 per cent hydrochloric acid (or 5 per cent sulphuric acid):

(1) There is a gradual drop in potential to a value of approximately −0·450 V. more active than the saturated calomel electrode during the first 30 min. of exposure. The initial change in potential with time is independent of applied load on the specimen.

(2) After the lowest potential has been reached there may be a gradual increase in potential of approximately 25 mV. or very little change at all. Short breaks in the potential-time curve may occur. These are seen as very rapid rises in potential to a more noble value and then return to 'normal'. The same result is obtained if the surface of the specimen is scratched.

(3) When the specimen fails, there is a sudden rise to a more noble potential.

(4) Cathodic polarization hastens time to failure. Anodic polarization prevents failure but causes pitting and etching attack.

We have a tentative explanation to offer for the observed potential changes. The reason that we qualify our explanation as 'tentative' is that we have additional work in progress to test our theory.

During the initial change in potential to a more active value, dissolution of metal proceeds fairly rapidly. The cathodic process is predominantly the reduction of selenous acid to selenium netal, which appears to be catalysed by the metal surface and deposits there. After the surface is covered by selenium, the potential remains steady or gradually becomes more noble, depending on the adhesion and porosity of the selenium layer. During this interval, if one scratches the surface and breaks the selenium layer exposing bare metal, a repetition of the deposition process occurs at this spot. This is reflected in a sudden rise in the potential and subsequent gradual return. We assume that hydrogen charging becomes the predominant cathodic process after the surface of the specimen is covered by selenium. As one increases the cathodic polarization, the time to failure after the selenium deposition process occurs is greatly decreased. The actual failure of the specimen is mechanical, that is, by hydrogen embrittlement. The reason for the sudden rise in potential is the sudden exposure of bare metal to the corrosive environment.

Another interesting environment is 5 per cent acetic acid with 0·1 per cent ammonium sulphide. The two 12 per cent chromium steels also crack in this environment by hydrogen embrittlement, but there is a major difference in the electrochemical behaviour. The steels still show a gradual potential drop in the more active direction, initially, reaching a value of about −0·520 V. more active than the saturated calomel electrode when cracking occurs. This time, however, there is no apparent change in potential when the specimen fractures or the surface is scratched. We believe the explanation here is that there is no competing cathodic process with hydrogen reduction and no insulating film on the metal surface; the mechanical breaking of the metal, therefore, will not cause a potential change.

These results confirm our opinion that the potential-time curve can be an effective tool in investigations into the cause of cracking of metals even when hydrogen embrittlement is suspected.

We wish to thank the Carpenter Steel Co. for the privilege of publishing this work and Dr. D. van Rooyen for his helpful information on stress corrosion cracking.

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Random Slip Model of Fatigue and Coffin's Law

Coffin[1] has shown that for a number of metals and alloys the relation between n the number of cycles to fracture and ε the plastic strain in each cycle is of the simple form:

$$n^{1/2}\,\varepsilon = \text{constant} \qquad (1)$$

This equation can be interpreted in a simple way on the model I have proposed[2]. According to this model the surface of the metal is roughened by a more or less random distribution of slip steps. Thus hills and valleys are formed on the surface, and subsequent slip tends to be concentrated in the valleys, roughly in proportion to their depths. The depth of each valley increases or decreases during fatigue in a step-wise manner analogous to a random walk. If $f(z, t)$ is the fraction of valleys (of width w) with depth between z and $z + dz$ after t cycles of fatigue, then $f(z, t)$ obeys a diffusion equation:

$$\frac{df}{dt} = D\,\frac{d^2f}{dz^2} \qquad (2)$$

The diffusion coefficient D is given by the expression $kbw\varepsilon$, where b is the slip vector and k is a geometrical factor of the order of unity.

The enhanced concentration of slip in the deeper valleys is taken into account by letting D be a function

of z. As a rough approximation we suppose that D increases linearly with z and put :

$$D = kbw\varepsilon\ (1 + \beta z/w)$$

where β is a constant of the order of unity.

The solutions of equation (1) with D a linear function of z have been given by Kepinski[3]. For large z they behave like $F \exp - z/\alpha t$, where α is the rate of increase of D with z, and F is a slowly varying function of z and t. In our case $\alpha = kb\beta\varepsilon$. For small values of t the exponential factor is very small, and no valleys attain any considerable depth. If Z is some large depth then a few valleys attain the depth Z after a number of cycles of the order $Z/15\alpha$. Thus if we take Z to be the depth at which a valley becomes an incipient crack the life-time of the specimen will be given by :

$$n = Z/15\alpha = Z/15\ kb\beta\varepsilon \qquad (3)$$

We now have to consider how this will change when ε is varied. We may anticipate that the value of Z will depend on the stress-level at which the specimen is fatigued, and thus on the value of ε. On the simple assumption that the plastic strain in each valley is linearly dependent on the depth z, we are led to suggest that the critical depth Z is given by an equation of the form :

$$\varepsilon Z/w = \varepsilon_c$$

where ε_c is a critical value of the plastic strain in a notch which leads to fracture. Combining this value of Z with equation (3) we obtain :

$$n\varepsilon^2 = w\varepsilon_c/15\ kb\beta \qquad (4)$$

which is equivalent to Coffin's equation.

From this derivation it might appear that this relation is a consequence of the simple linear dependence of D and ε on z. However, the calculation can be carried through for more general forms of dependence of D and ε and a relation of the form (4) is still obtained.

We may derive a value for ε_c from a comparison of equations (4) and (1). The experimental value of the constant in (1) is near unity, and we may insert the values of b and w which were used in the previous note[3], $w = 10^{-4}$ cm., $b = 5 \times 10^{-7}$ cm. The value of ε_c so obtained is $15\ b/w$ or $7{\cdot}5 \times 10^{-2}$. This is of course an extremely rough estimate ; but it appears to be a likely order of magnitude for a local plastic strain sufficient to propagate a crack.

One remarkable feature of Coffin's equation is that it is still valid for $n = \frac{1}{4}$, that is, the real strain in fracture by uni-directional strain obeys the same relation. This suggests that the formation of small notches by slip may be relevant in simple ductile fracture as well as in fatigue.

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RADIATION CHEMISTRY

Formation of Activated Hydrogen Peroxide by Irradiation of Water with α-Rays

When water is exposed to ionizing radiations the radicals H and OH are produced and these can combine either with each other to give the molecular products H_2 and H_2O_2, or with solutes[1]. In regions of high energy loss (such as α-particle tracks) the local concentration of radicals is sufficiently high for combination between them to occur sooner than reaction with the solute. Consequently, while the chemical effects of X-rays on aqueous solutions are brought about chiefly by radical reactions, those of α-rays are chiefly by molecular reactions. However, in α-irradiations there are δ-rays associated with the α-particle tracks and these always introduce a small fraction of relatively sparsely ionizing radiation which prevents any absolute distinction being made between the effects of X- and α-irradiations.

While studying the effect of ionizing radiations on solutions of proteins, we noticed that polonium α-rays gave rise to changes which were qualitatively different from those brought about by X-rays[2], but which could not be obtained by treatment with hydrogen peroxide even when this was added to a final concentration of 0·6 *M*, a thousand times greater than that produced by the α-rays in the water. To elucidate this phenomenon we have compared the effects of X- and α-radiations on solutions of certain amino-acids. With tryptophan in particular, there is clear evidence that an oxidizing agent of reactivity greater than that of normal hydrogen peroxide is formed by the α-rays.

The α-ray source was 100 mc. of polonium-210, deposited on a gold-foil disk, and mounted so that it could be screwed down over an open vessel containing the solution. The whole arrangement was within a container filled with nitrogen. Nitrogen was bubbled through the solution for 30 min. before irradiation and blown over it during irradiation, while the solution was being stirred with a glass-covered rotating magnet. The source of X-rays was a Westinghouse deep-therapy set, run at 220 kV. with no filter. Dose-rates were measured with ferrous sulphate solutions held in the same position as the experimental solutions. The α-radiation was delivered at the rate of 20,000–45,000 rads/hr. ($G = 5{\cdot}6$) and the X-radiation at 6,640 rads/min. ($G = 15{\cdot}6$). The tryptophan, from a commercial sample of high purity, was used at a concentration of 2×10^{-4} *M* in water redistilled in an all-glass apparatus.

Changes in the ultra-violet absorption spectra (differences in extinction coefficient between the irradiated and unirradiated solutions) produced by X- and α-rays are shown in Fig. 1. The loss of absorption at 280 mμ is in both cases connected with loss of the tryptophan chromophore (the indole ring), but the products of the two radiations are evidently different. Hydrogen peroxide at a final concentration of 8×10^{-3} *M* for 3 days produced no detectable changes. We have confirmed the spectral changes produced by treating tryptophan with ozone[3], and find that these closely match the changes produced by α-irradiation. However, the conditions of our experiments preclude the formation of ozone, and even if the irradiations had been carried out in an atmosphere of pure oxygen the yield of ozone would need to be unreasonably large ($G > 50$) if it were to

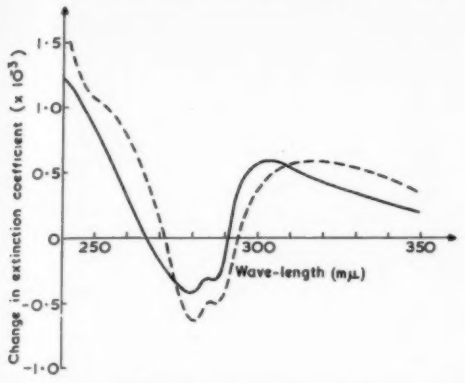

Fig. 1. Differential absorption spectra (difference between extinctions of irradiated and unirradiated samples) for 2×10^{-4} *M* tryptophan following X-irradiation (66,400 rads) and α-irradiation (59,000 rads). Both irradiations were under nitrogen
– – –, α-irradiation; ——, X-irradiated

produce the observed effects. Treatment of the tryptophan with chemically produced OH radicals (from hydrogen peroxide and ferrous sulphate) caused changes in the absorption spectrum which, as expected, closely matched those caused by X-irradiation.

A direct measurement of the indole ring concentration, by the method of Spies and Chambers[4], showed (Fig. 2*a*) that the concentration fell more rapidly on α-irradiation (with an initial yield per 100 eV. of $G^{\alpha}_{\text{-indole}} = 1{\cdot}8$) than on X-irradiation ($G^{X}_{\text{-indole}} = 0{\cdot}92$). However, estimation with Nessler's reagent of the amount of ammonia released (by deamination of the tryptophan) showed (Fig. 2*b*) that in this respect the radiations were equally efficient ($G^{\alpha\text{-}X}_{NH_3} = 1{\cdot}8$). Addition to the solution of 0·1 per cent ribonuclease, a protein containing no tryptophan, protected tryptophan against the radiations; but, whereas in the α-irradiated solution the effective dose to the tryptophan moiety was reduced to about 70 per cent of the dose given to the whole solution, in the X-irradiated solution, the effective dose to the tryptophan was reduced to 35 per cent. These experiments indicate that the molecular product formed in water by α-rays is more reactive and more selective than the radicals produced by the X-rays. Ribonuclease can act as an efficient scavenger for OH radicals, which react readily with all the amino-acids constituting the protein, but it does not compete so effectively for the α-radiation products.

The results show that α-irradiation of water yields a highly oxidizing product which reacts with particular readiness with the indole ring of tryptophan, as

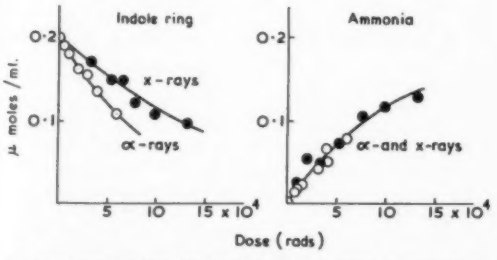

Fig. 2. Analysis of irradiated solutions of 2×10^{-4} *M* tryptophan after various doses of X- and α-radiation
○, α-irradiated; ●, X-irradiated. *a*, indole ring concentration; *b*, ammonia concentration

does ozone[3]. However, direct experiment shows that hydrogen peroxide has insufficient reactivity, and the experimental conditions rule out ozone as the product in question. Furthermore, the yields are too high for the product to be the radical HO_2 ($G^{\alpha}_{HO_2} = 0{\cdot}15$, ref. 1). We suggest that the product is hydrogen peroxide formed, by the combination of two OH radicals, either in an excited state, or, less probably, in a reactive isomeric form. Since it reacts with a substrate at a concentration of 10^{-4} *M* the life-time of this intermediate must be at least 10^{-5} sec. This eliminates the possibility that a singlet state is involved. But although we have not been able to find any work establishing a triplet state of hydrogen peroxide, the existence of this state is probable on general grounds.

The formation by α-rays of activated hydrogen peroxide as an intermediate of relatively long life-time would create a situation in which α-rays could be more effective than X-rays. A molecule, such as a protein containing tryptophan, would be particularly susceptible to attack in a complex mixture, such as exists in a cell, where the OH radicals formed by X-rays would be used up to a greater extent in reaction with the other substances present. The formation of an activated form of hydrogen peroxide could in this way be one of the causes making the biological efficiency of densely ionizing radiations (for example, α-rays) greater than that of sparsely ionizing radiations (for example, X-rays) though other factors are certain to be involved[5].

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Simultaneous Determination of Scandium and Dysprosium by Neutron Activation followed by γ-Ray Spectrometry

A neutron activation analysis method has been devised for the non-destructive, simultaneous determination of scandium and dysprosium.

It is known that when natural scandium and dysprosium are irradiated with neutrons, scandium-46*m* (19·5 sec.) and dysprosium-165*m* (1·25 min.) are formed, respectively. The method of analysis by the use of these nuclides has never been described in the literature examined.

In the present work, the sample (3–80 mgm.) is placed in the nuclear reactor *JRR*-1 for 30 sec. to be irradiated with neutrons at a flux of 4×10^{10} neutrons/cm.²/sec. (when operated at 5-kW. level), cooled for 10 sec., counted for 30 sec., again cooled for 30 sec. and counted for 30 sec. The counting apparatus used is a 256-channel pulse height analyser to which a 2-in. thick, well-type sodium iodide crystal is attached. In the first counting 128 channels in the analyser are used, and in the second counting the rest used. In the resultant γ-spectrum the height of photopeak due to 0·142-MeV. γ-ray of scandium-46*m*

and that due to 0·108-MeV. γ-ray of dysprosium-165*m* are measured, respectively.

The relations between photopeak height and the amount of scandium or dysprosium have been obtained by use of standards.

Table 1. Examples of the Coefficient of Variation and the Corresponding Size of Photopeak

Sample	Coefficient of variation of single analyses (per cent)	Average photopeak height (counts per channel)	Average area of photopeak (counts)
Scandium, 3·07 μgm.	3·8[a]	1·88 × 10³	4·3 × 10⁴
Dysprosium, 1·62 μgm.	2·1[a]	4·56 × 10³	7·4 × 10⁴
	9·5[b]	2·60 × 10³	4·7 × 10³

a, Obtained from the first counting; *b*, obtained from the second counting.

The examples of the coefficient of variation of single analyses have been obtained by use of five scandium samples, each of which includes 3·07 μgm. of scandium, and of four dysprosium samples, each of which includes 1·62 μgm. of dysprosium. The coefficients estimated are shown in Table 1 with the related data.

Results of analyses are shown in Tables 2 and 3.

Table 2. Results of Analyses for Synthetic Samples

Sample	Scandium added (μgm.)	Dysprosium added (μgm.)	Scandium found[a] (μgm.)	Dysprosium found (μgm.)	
				(*a*)	(*b*)
A	0·98	1·62	0·98	1·62	1·77
B	0·98	1·62	0·97	1·59	1·53
C	0·98	1·62	0·92	1·53	1·34

a, Obtained from the first counting; *b*, obtained from the second counting.

Table 3. Results of Analyses

Sample	Amount of sample (mgm.)	Scandium found (p.p.m.)	Dysprosium found (p.p.m.)
A (Soil)	49·6	19	<3
B (Monazite sand)	3·26	< 5 × 10¹	6·7 × 10³
C (Orthite, Korea)	3·55	<10	8·1 × 10¹
D (Columbite, Japan)	47·5	26	<8
E (Yttrium oxide)	84·5	<0·4	<2

I am indebted to Prof. Shoji Makishima for helpful suggestions, to Dr. Toshio Nakai for permission to use laboratory facilities, and to Mr. Fumio Aoki for constant encouragement.

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CHEMISTRY

Nature of Fluids in the Hypercritical Region

In the opinion of Andrews[1] the question of the appropriate nomenclature to be applied to a fluid at temperatures and pressures above the critical point "does not admit . . . of a positive answer. . . . Carbonic acid at 35·5°, and under 108 atmospheres pressure, stands nearly midway between the gas and the liquid; and we have no valid grounds for assigning it to the one form of matter any more than to the other". The absence of any sharp visible transition when a substance is taken from the gas state below the critical temperature to the liquid state, also below the critical temperature, by a route which does not enter the two-phase region of the p,v diagram (that is, cross the two-phase line on the T,p diagram), gave rise to the concept of the 'continuity of gaseous and liquid states'; and this has, perhaps, discouraged inquiry into the structural changes which must occur on passing from a dilute gas to liquid. Suggestions concerning the distinction between gaseous and liquid states of hypercritical fluids were made by Thiessen[2] in 1897, and later by Eucken[3] and by Plank[4]. In recent years, interest in this subject has revived, and several suggestions have been made[5–8] that the hypercritical region of the T,p diagram should be divided into areas corresponding to 'gas-like' and 'liquid-like' states of matter by what Bernal[6] proposes should be called the 'hypercritical line'. The present communication discusses the criteria which have been suggested for making this subdivision.

Smith[5] has directed attention to the fact that whereas, below the critical temperature, the coefficient of thermal expansion of a substance at constant pressure when plotted against temperature goes to infinity at the boiling point, above the critical temperature it has a maximum. Since real gases are usually characterized by $\partial^2 v/\partial T^2 < 0$ and liquids by $\partial^2 v/\partial T^2 > 0$, this maximum may be regarded as separating gas-like and liquid-like states of the hypercritical fluid. It is found empirically that this maximum occurs at roughly $v_{max.} \approx v_c$, although $v_{max.}$ tends to fall with increasing pressure[7]. In a recent lecture[6], Bernal suggests that since a sharp maximum is observed when C_p is plotted against temperature at constant pressure[8,9], and since such maxima in general reveal the existence of higher-order phase transitions, the locus of this maximum in T, p co-ordinates should be called the hypercritical line separating 'incoherent' from 'coherent' states. Bernal suggests, on geometrical grounds, that this transition should occur at a volume slightly greater than three times the close-packed volume of the molecules. Kaganer[7] has discussed various ways of dividing up the hypercritical region, and has shown by analysis of the available data that the two methods described above lead to different boundary lines, although they are both smooth prolongations of the saturated vapour pressure curve. He appears to favour the locus of $(\partial C_p/\partial T)_p = 0$ as the most appropriate boundary between liquid and gaseous states, on the grounds that it is the "most natural continuation" of the vapour pressure curve. A similar view is expressed by Jones and Walker[8].

The close connexion between the criteria suggested by Smith and by Bernal is revealed by the thermodynamic equation $(\partial^2 v/\partial T^2)_p = -(1/T)(\partial C_p/\partial p)_T$, which shows that the locus of maxima in $(\partial v/\partial T)_p$ as a function of T will follow the same path on the T, p diagram as the locus of maxima in C_p as a function of p. This relationship is best illustrated by a three-dimensional model (Fig. 1) in which C_p for argon is shown as a function of T and p. Smith's criterion corresponds to choosing the locus of maxima of the plane sections of the model at constant T (line i), whereas Bernal's is the locus of maxima of plane sections at constant p (line ii). If, however, we choose to employ C_p as the thermodynamic function in terms of which we wish to distinguish the two forms of hypercritical fluid, then the line of the ridge

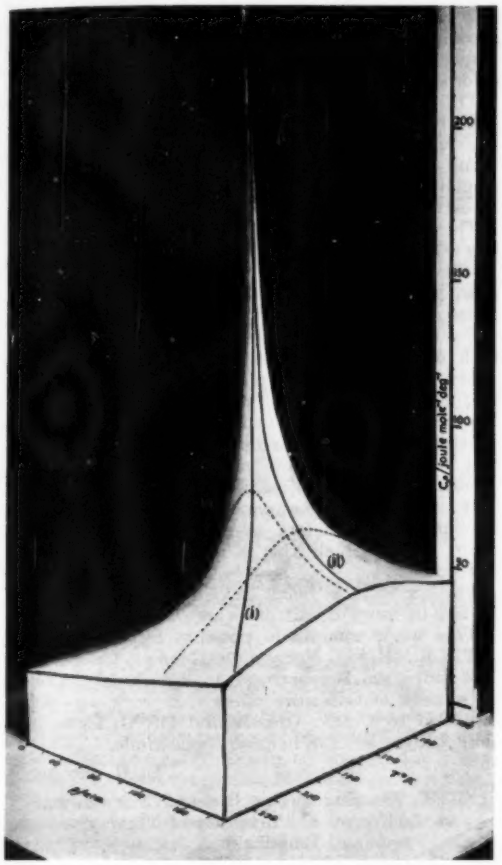

Fig. 1. C_p for argon as a function of temperature and pressure above the critical point. Data are from refs. 8 and 9.
Line (i): locus of $(\partial C_p/\partial p)_T = 0$
Line (ii): locus of $(\partial C_p/\partial T)_p = 0$

or watershed in the three-dimensional model has a strong claim to be used to define the hypercritical line in the T,p plane. It turns out, however, that although the watershed lies between (i) and (ii), there is no general mathematical criterion which identifies its position uniquely; and, even when the watershed can be defined, its locus on the T,p plane depends on the relative scales employed for T and p. (We are indebted to Dr. T. H. K. Barron for investigating the mathematical definition of the watershed and for pointing out that its locus cannot be defined generally.)

It seems, therefore, that a unique definition of the hypercritical line should not be attempted, but that the physical characteristics of the transition are better described by talking of a 'hypercritical transition region' commencing as a smooth extrapolation of the saturated vapour pressure curve at the critical point, but fanning out at higher temperatures and pressures, and having as its most intense regions the area between the lines defined by $(\partial^2 v/\partial T^2)_p = 0$ (or $(\partial C_p/\partial p)_T = 0$) and $(\partial C_p/\partial T)_p = 0$. The transition is very intense near the critical point so that, for some distance above it, the two lines are barely distinguishable.

It is perhaps also worth noting that, whereas Bernal has invoked a geometrical model to predict that the transition region should correspond to volumes approximately three times the close-packed volume, an essentially similar conclusion follows directly from the van der Waals' equation, for which $(\partial^2 v/\partial T^2)_p = 0$ when $v_{max.} = 3b = v_c$. It is not clear to us why this result should follow from the van der Waals' model, and it is probably a fortuitous mathematical result; nevertheless, it seems empirically to provide a reasonable approximation to the position of the hypercritical transition region.

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Effect of Corona Discharges on Polyethylene

It is known that organic insulation materials deteriorate when subjected to corona discharges. Although many workers have studied the breakdown of insulation materials under the influence of corona, particularly for the purpose of obtaining standardized testing procedures, few have been concerned with the fundamental physical and chemical processes involved.

In the present work *n*-eicosane was used as a model compound for polyethylene. The paraffin was subjected to corona discharges in air, oxygen and carbon dioxide. The experiments were carried out in a cell consisting of two superimposed glass plates spaced 3–5 mm., the outer surfaces of which were made conducting. The paraffin was cast as a thin layer on one of the inner surfaces. The voltage source was a 20-kV. r.m.s. 50 c./sec. transformer.

After exposure to corona discharges for 25–50 hr., the paraffin, which was now partly oxidized, was treated with lithium aluminium hydride in tetrahydrofuran, after which the resulting alcohols were separated by gas chromatography on an 'Apiezon' grease column at 210° C. The chromatogram is shown in Fig. 1.

As will be seen from Fig. 1, only the *n*-mono-alcohols are present. It will further be seen that the

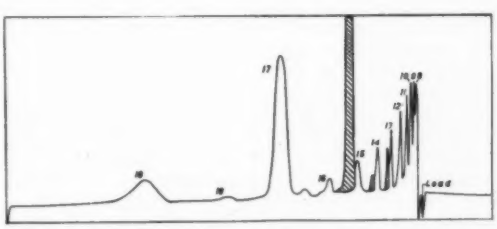

Fig. 1. Gas chromatogram of *n*-mono-alcohols with carbon numbers 8–19 derived from oxidation products of *n*-eicosane. The shaded peaks are *n*-eicosane and its impurities

amount of *n*-heptadecanol ($C_{17}H_{35}OH$) is without comparison the largest. It must therefore be concluded that the discharges cause a scission of a C—C bond, and that the bond between the third and fourth carbon atom is most likely to be attacked.

From mass spectrometer work[1] it is known that this is exactly what happens in the mass spectrometer, where the molecule is ionized after which a charged fragment splits off. The similarity of the fragmentation pattern strongly suggests that the same mechanism is operative also under the influence of corona discharges.

When an alternating voltage is applied to the cell, a discharge will pass when the potential between the two surfaces exceeds the breakdown voltage of the air-gap. In air the breakdown gradient at 1 atm. is 30 kV./cm. and the calculated mean energy of electrons 3·2 eV., whereas the ionization potential for most *n*-paraffins is 10–11 eV. (R. F. Grossman and W. A. Beasley[2] have suggested a mechanism for the breakdown of polyethylene by corona discharges, based on the assumption of the presence of 28 keV. electrons, which in my opinion is incorrect.)

Assuming a Maxwellian distribution of the energy of the electrons around the mean of 3·2 eV. it can be calculated that 4·2 per cent of them possess energies of 11 eV. or more. As only a minute fraction of the energy input to the cell is involved in chemical transformations of the paraffin, the number of electrons possessing this energy is sufficient to explain an ionization mechanism for the fracture of the molecules.

By varying the experimental conditions, further evidence was furnished in favour of this mechanism, as the extent of bond scission was found to be proportional to the number of electrons that collide with the paraffin surface.

Polyethylene film was then exposed to corona discharges in the same cell. After 25–50 hr. treatment the film was treated with lithium aluminium hydride in tetrahydrofuran. The soluble material was now expected to be diols and was difficult to separate by gas chromatography. Successful separation was accomplished after converting the diols into their trimethylsilyl ethers by treatment with trimethylchlorosilane in pyridine.

The chromatogram is shown in Fig. 2.

The presence of large amounts of ethylene glycol was not unexpected as crystals of oxalic acid were found on the surface of the film in most experiments. Oxalic acid was also found and characterized by other investigators[2,3].

The suggested ionization mechanism applied to polyethylene leads to the following reaction scheme.

When polyethylene is subjected to corona discharges in an oxygen-containing atmosphere, molecules are ionized and a positive ion splits off the molecule end, leaving a free radical which combines with oxygen to an alkoxy radical. This radical is again ionized, splitting off preferably a two-carbon ion, and this process is repeated along the chain. The radical ion which is split off the chain is further oxidized to the diol, the diacid and to carbon dioxide and water, which were also detected as reaction products. Instead of starting at the molecule end only, it can also be assumed that chain end radicals are formed by random scission of the polyethylene chain, after which the process continues as described.

Further work is being carried out and will be reported in more detail elsewhere.

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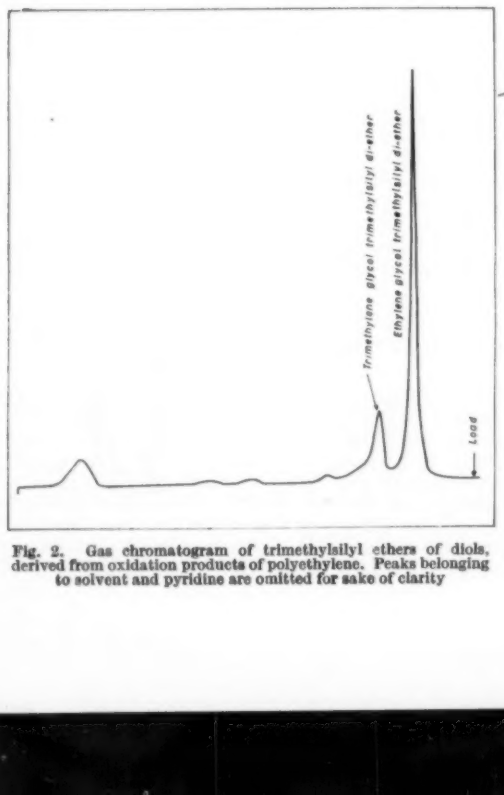

Fig. 2. Gas chromatogram of trimethylsilyl ethers of diols, derived from oxidation products of polyethylene. Peaks belonging to solvent and pyridine are omitted for sake of clarity

An Ultra-sensitive Ionization Detector for Permanent Gas Analysis

The development of a high-sensitivity detector responsive to all elements or compounds in the chromatographic analysis of gaseous mixtures is of importance in the determination of substances present in micro amounts.

A radioactive ionization-type detector similar to the argon detector described by Lovelock[1] was used with helium carrier gas. The metastable helium atoms (19·8 eV.) formed are capable of ionizing, with the exception of neon, all elements and compounds. This means that the technique may be used in the chromatographic analysis of permanent gases where the ionization potentials range from 12 to 16 eV.

Using a molecular sieve 5*A* column of 4 mm. diameter and 30 cm. long, it is possible to determine less than 0·001 μl. of hydrogen, argon, oxygen, nitrogen, methane and carbon monoxide in less than 2 min. Up to 1 μl. of gas can be determined before the detector is saturated. The column is operated at room temperature, except when oxygen–argon mixtures are analysed, when cooling to −80° C. is re-

quired. The limit of detection is approximately 10^{-13} mole of component per ml. of carrier gas.

It is essential to purify the helium carrier gas from all substances other than neon, otherwise too high a background current occurs and the system becomes saturated. It is difficult at this stage to state the permissible impurity level. The ratio of ionization currents for 1 v.p.m. added impurity (nitrogen, oxygen, methane, argon) against the 'pure' helium is 1·05. This is better than the value obtained by Jesse and Sadauskis[2] of 1·003.

The helium purification system used is a combination of physical and chemical methods, comprising molecular sieve at 20° and −196° C., titanium at 1,000° C. and 'Hopcalite' at 400° C.

A detailed report on the application of this technique to the analysis of permanent gases is to be published.

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Proton Magnetic Resonance of the Water Phase of Gelatin Gels

Results have recently been reported concerning the proton magnetic resonance line-width of the water phase of starch gels[1]. I have examined the solvent line-width of gelatin gels at 16·7 Mc./sec. in a field of 3,910 gauss with the spectrometer now in use at this laboratory. The samples were contained in soda glass tubes of 1·2 mm. inside diameter. Sample spinning at about 1,200 r.p.m. was employed. The instrumental resolution was 0·12 p.p.m. The measurements were carried out at +20° C. The gels were made by solution at 85–90° C. of commercial gelatin in distilled water. The most concentrated gel (0·2 gm. gelatin per ml. water) was also examined with various concentrations of sodium chloride.

The line-width as a function of gelatin concentration is given in Fig. 1 (upper curve). The line-width increases rapidly up to about 6 c./s. at 0·05 gm./ml. At higher concentrations of gelatin the increase in line-width was small. On addition of sodium chloride, the line-width decreased considerably, but did not reach the value for water. As suggested in the article on starch gels[1], this upper limit of line-width of the aqueous phase may be due to the molecular mobility of macro-molecules. Approximate measurements of T_2 by the wiggle regrowth method[2] indicated that T_2 decreased from about 1·5 sec. for distilled water (in equilibrium with air oxygen) to about 0·05 sec. for the most concentrated gel. Approximate measurements were also made on T_1 by the progressive saturation method[3]. T_1 decreased from about 2 sec. in distilled water to about 0·15 sec. in the most concentrated gel (0·2 gm./ml.).

Work on different types of biological gels is being continued, and the proton resonance of organic macro-molecules is being investigated on gels in heavy water (D_2O).

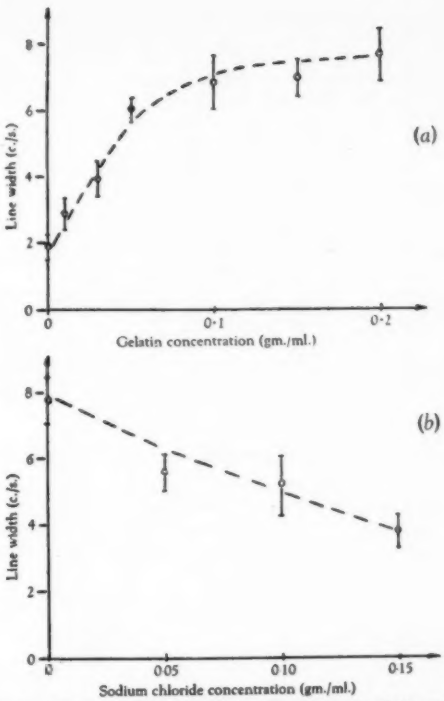

Fig. 1. Proton magnetic resonance line-widths of gelatin gels. (a) Line-width as a function of gelatin concentration in distilled water. (b) Line-width of a gelatin gel containing 0·2 gm. gelatin/ml. water as a function of concentration of sodium chloride in the gel

This work was supported by grants from the Swedish State Medical Research Council and Swedish National Association against Heart and Chest Diseases.

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BIOCHEMISTRY

Analysis of Human Leucocyte Deoxyribonucleohistone by Immunoelectrophoresis on Cellulose Acetate

The internal composition of the nucleus is still unrevealed to a large extent despite many recent advances. The work described here constitutes a new approach to the problem made possible by a combination of histochemistry and immunoelectrophoresis on cellulose acetate membranes. Kohn[1] first introduced cellulose acetate as a supporting membrane for zone electrophoresis, and Consden and Kohn[2] showed that this material was elegantly suited to the entire process of immunoelectrophoresis. In this laboratory the techniques were found to be easily reproducible[3] and are now being used to study the human leukæmic leucocyte, with particular reference to the nucleus.

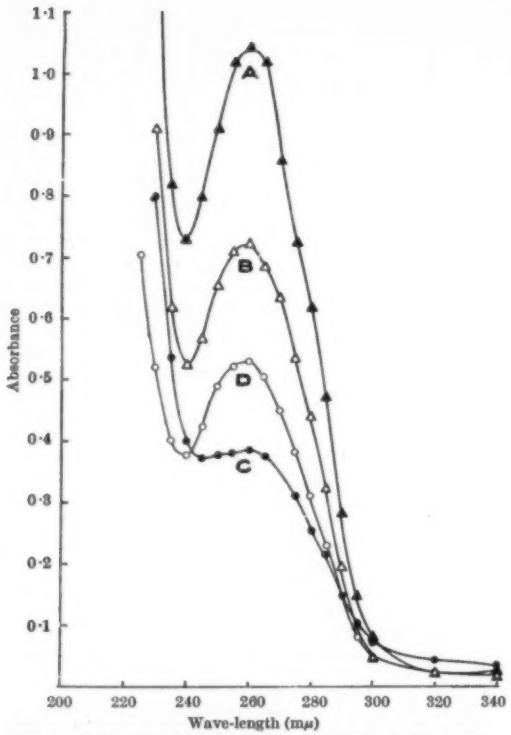

Fig. 1. Ultra-violet absorption studies of an extract of leukæmic leucocytes. Curve *A* is the spectrum of the whole, fresh extract; curve *B* is the spectrum of the material remaining in the sac after overnight dialysis against copious amounts of 0·1 *M* glycine; curve *C* is the spectrum of that portion of the extract which remains soluble after dialysis against 0·15 *M* sodium chloride; curve *D* is the spectrum of the material which is insoluble in 0·15 *M* sodium chloride after it has been re-dissolved in 0·1 *M* glycine. All samples were studied at a dilution of 1 : 40

In a typical experiment, leucocytes which have been obtained by venepuncture from patients with leukæmia are separated from the erythrocytes after accelerating the sedimentation of the latter by the addition of 'Dextran'. Serum proteins are removed by washing the leucocytes three times with 0·1 *M* sodium chloride–0·1 *M* sodium citrate alternating with periods of centrifugation for 1 min. at about 1,500 r.p.m. Two more washes with 0·05 *M* sodium citrate help leach soluble components from the cytoplasm of the now presumably non-viable cells and promote the ultimate dissolution of the nucleus[4]. Treatment with 0·1 *M* glycine then lyses the cells and puts the entire nucleus into solution as shown by phase microscopy. (This method of extracting deoxyribonucleoprotein was suggested by Dr. Daniel Mazia; further discussion of its applications will appear elsewhere.) After extraction has continued overnight, the preparation is freed of sediment by centrifugation at 20,000 r.p.m. for 30 min. All steps are performed at 4° C. and all solutions contain 0·001 *M* ethylenediamine tetraacetate as a further precaution against the action of the enzyme deoxyribonuclease.

The extracts are virtually colourless, slightly opalescent, and not viscous despite the presence of 0·5–1·5 mgm. per ml. of deoxyribonucleic acid as measured by the Dische diphenylamine reaction. About five to eight times as much protein is present on a weight basis as measured by the Biuret reaction calibrated with crystalline bovine albumin. Typical ultra-violet absorption studies of an extract of leucocytes from a patient (Dal.) with acute lymphocytic leukæmia are shown in Fig. 1. Curve *A* shows that the fresh extract has a peak absorption at 260 mμ and a shoulder at 280 mμ; curve *B* shows that the majority of the extract is non-dialysable; curve *C* shows that most of the ultra-violet absorbing material is insoluble in physiological saline; and curve *D* shows that this insoluble material can be redissolved in 0·1 *M* glycine to give an absorption spectrum characteristic of a nucleoprotein. It is apparent that the extracts are extremely rich in a high molecular weight deoxyribonucleic acid and associated proteins.

Electrophoresis was performed on cellulose acetate membranes supported in a micro-electrophoretic apparatus[3]. Separations were carried out for 90 min. at a constant voltage of 150 V., which gave an average current of 1·5 m.amp., using a barbital buffer *p*H 8·6 with an ionic strength of 0·075. At the conclusion of the electrophoretic separation the strips were dried and stained for histone by the method of Alfert and Geschwind[5] and for deoxyribonucleic acid by the Feulgen technique.

Fig. 2 shows the electrophoretic separation of an extract of cells from a patient (Peg.) with chronic granulocytic leukæmia. Strip *A* has been stained for histone and shows two prominent bands migrating toward the anode, one more rapidly than the other. Strip *B* has been stained for deoxyribonucleic acid and shows only a single band migrating also toward the anode. Strip *C* was divided length-wise into two parts after the electrophoretic separation and then stained, *C*1 for histone and *C*2 for deoxyribonucleic acid. It is clear that all the deoxyribonucleic acid is associated with the faster-moving histone band. Examination of the original strips shows several bands of protein migrating toward the

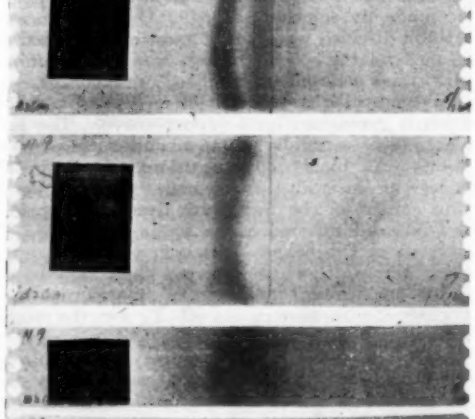

Fig. 2. Replicate electrophoretograms of an extract of leukæmic leucocytes. Strip *A* has been stained for histone, strip *B* has been stained for deoxyribonucleic acid, strip *C* has been divided length-wise after electrophoretic separation and *C*1 stained for histone, *C*2 for deoxyribonucleic acid. (The anode is at the left)

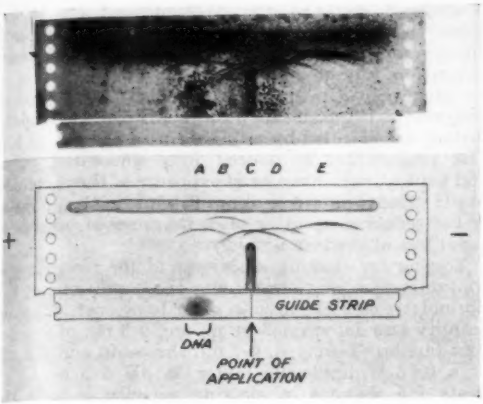

Fig. 3. Immunoelectrophoretic analysis of an extract of leukæmic leucocytes using homologous antiserum. The narrow guide strip along the bottom has been stained for deoxyribonucleic acid, but the Feulgen-positive material reproduced poorly in the photograph. A line drawing of the experiment has been added for extra clarity

cathode which stain very faintly with the histone stain, but the significance of these remains to be determined.

Antisera were prepared by emulsifying cell extracts with equal parts of Freund's complete adjuvant and injecting the mixture subcutaneously into a rabbit at weekly intervals for four weeks. Serum was collected after the sixth week and used for immunoelectrophoretic work on the composition of the extracts. The technique was that described by Consden and Kohn[2] with the following modification. Immediately after the electrophoretic separation a narrow guide-strip was cut from one edge of the membrane so that, by appropriate staining, it might serve to identify the location and nature of the substances present on the larger strip. Antiserum was then applied to the opposite edge of the latter and immuno-diffusion allowed to occur at room temperature for two days, following which the precipitin lines were stained with nigrosine.

In Fig. 3 is depicted a typical immunoelectrophoretic and histochemical analysis of an extract of leucocytes from patient Peg. using an antiserum to the extract prepared as described above. The precipitin line under the letter *A* was clearly seen to be associated with that portion of the electrophoretic separation which gave a positive stain for deoxyribonucleic acid by the Feulgen reaction, thus indicating the location of the deoxyribonucleic acid–histone complex described in the electrophoretic work mentioned above. Line *B* and possibly line *C* may well represent precipitin reactions due to the presence of slower moving histones, but definitive evidence is not yet available. The number of precipitin lines indicates the presence of at least five antigenically distinct components in the extract, none of which reacts with potent serum from a rabbit sensitized to human serum.

It remains to be determined which portion of the deoxyribonucleic acid–histone complex is responsible for the antigenicity demonstrated. Other experiments under way include efforts to determine the total number of basic proteins present, to identify the chemical nature of all the antigenically distinct components, to demonstrate possible organ and species differences, and to compare the patterns of leukæmic cells with those of normal cells.

This work was supported by grants from the Public Health Service (*C*-5060), the Martha and Spencer Love Foundation, and Mrs. Sherley W. Morgan. I am indebted to Dr. Daniel Mazia for frequent consultation on this project, to Mary Morrison for valuable technical assistance, and to Dr. David A. Wood, director of the Cancer Research Institute, for his continued interest and support.

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Reduction of Nitroso Group in Heterocyclics

In search of a better and convenient method for the reduction of the 5-nitroso group of substituted pyrimidines, an intermediate for the synthesis of substituted pteridines, required for the synthesis of antagonist of folic acid, as a possible tumour-inhibiting agent (cf. aminopterin and amethopterin), nickel–aluminium alloy in alkaline solution is found to be a very suitable and convenient reagent. Besides the use of nickel–aluminium alloy as catalyst for hydrogenation of various unsaturated compounds, it has been used most successfully in alkaline medium for the reduction of carbonyl groups to the corresponding hydrocarbons or carbinols[1], for the exchange of halogens by hydrogens[2], for the reduction of isolated, conjugated or cyclic double bonds[3], for the hydrogenolysis of alkoxy or methylene dioxy groups in aromatic compounds[4], various sulphur compounds[5] and furan derivatives[6].

It has been reported earlier that the reduction of the 5-nitroso group of the various substituted pyrimidines can be carried out with sodium hydrosulphite, and the corresponding amino derivatives were isolated as their bisulphite salt[7–9]. Attempts were made to prepare 2 : 4 : 5-triamino-6-hydroxy pyrimidine from the corresponding 5-nitroso derivative by the reduction of the latter with sodium hydrosulphite, both in neutral and alkaline medium ; but the yield was found to be unsatisfactory, possibly due to the quality of the hydrosulphite used for this purpose. The present method using nickel–aluminium alloy in alkaline medium, as the reducing agent, was found to be very satisfactory.

2 : 4-Diamino-6-hydroxy pyrimidine was converted into the corresponding 5-nitroso derivative with sodium nitrite in cold hydrochloric acid, which was isolated as an orange-coloured precipitate. Reduction was carried out in warm (50–55° C.), dilute sodium hydroxide solution by adding powdered nickel–aluminium alloy (50 per cent) in portions with vigorous stirring until the red colour of the nitroso compound was completely bleached. The mixture was immediately cooled and filtered from the unreacted alloy when the smell of ammonia was noticed. The cooled filtrate was acidified with sulphuric acid and kept in the cold overnight, when the sulphate of the 2 : 4 : 5-triamino-6-hydroxy pyrimidine was crystallized out in fairly good yield. The base was isolated from the corresponding sulphate with sodium bicarbonate and

crystallizing the precipitated base from water, the compound was identified by its analysis as well as the ultra-violet absorption spectrum.

Using the same procedure, 2 : 4 : 6-trihydroxy pyrimidine, 2 : 4-dihydroxy-6-amino pyrimidine and 2-amino-4 : 6-dihydroxy pyrimidine were converted into their 5-nitroso derivatives, which in turn were smoothly reduced to the corresponding amino derivatives in very good yield. It was, however, observed that 2 : 4-dihydroxy-6-methyl pyrimidine and 2-hydroxy-4 : 6-dimethyl pyrimidine could not be converted into their 5-nitroso derivatives under similar conditions. A steric effect possibly is the reason for this failure.

The details of the work will be published elsewhere in due course.

We thank Dr. Subodh Mitra, director of this Centre, for his advice and constant encouragement during the progress of the work.

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Selective Inactivation of the Plasminogen Contaminant in Thrombin

The contamination of preparations of thrombin and fibrinogen with plasminogen, the precursor of the fibrinolytic enzyme, has always presented great difficulties in the measurement of fibrinolytic activity.

When clots prepared from such 'contaminated' substances are used for the assay of fibrinolytic material having activator activity, the resulting lysis will be due to (*a*) activity of the added plasmin itself, and (*b*) activation of the contaminant plasminogen in the clot.

While plasminogen contamination of fibrinogen can be reduced to a minimum by applying Laki's procedure[1] three times, no satisfactory procedure has been available to free thrombin preparations from plasminogen. Milstone's method[2] and an alcohol-fractionation adopted by us (unpublished) result in yields of less than 1 per cent and are therefore impractical. The procedure described here involving incubation of thrombin with the reducing agent, 2-mercaptoethylamine, and its subsequent removal by dialysis results in loss of potential fibrinolytic activity with no impairment of clotting ability.

The contents of 1 vial of bovine 'Thrombin Topical' (Parke, Davis and Co.) containing 10,000 N.I.H. units are dissolved in 10 ml. of 0·5 *M* 2-mercaptoethylamine hydrochloride (Calif. Found. for Biochem. Res.) previously adjusted to *p*H 7·4. After 10-min. incubation at room temperature, the solution is dialysed at 4° C. against 10 litres of $1{\cdot}5 \times 10^{-4}$ *M* imidazole buffer, *p*H 7·0. After 5 hr. the dialysate is changed and the dialysis continued for 16 hr. Since the two dialyses amount to a million-fold dilution of the original 2-mercaptoethylamine concentration, any amount remaining in the solution is, for all practical purposes, negligible. At the end of dialysis the bag contents (now at *p*H 6·3 and slightly turbid) are clarified by adjustment of the *p*H to 7·4. The preparation is poured into siliconized tubes and kept frozen. Even brief exposure of the thrombin to *p*H values below 5 or above 9, while in the presence of the reducing agent or after its removal, results in rapid loss of clotting activity.

Testing for clotting activity and for plasminogen contamination was done under the conditions of the fibrinolytic assay as used in our laboratory[3]. Clotting activity was determined by mixing 0·3 ml. of 0·6 per cent human fibrinogen, 0·1 ml. thrombin and 0·2 ml. of a 0·05-*M* imidazole buffer at *p*H 7·2 at 45° C. Tests for changes in clotting activity due to the procedure were carried out by determining the clotting time of the above mixture using thrombin treated, and untreated, with 2-mercaptoethylamine, at several dilutions (Table 1). All dilutions of thrombin were made in imidazole buffer. Treatment with 2-mercaptoethylamine resulted in no impairment of clotting activity. Testing for the plasminogen contamination of thrombin samples was carried out by determination of the lysis-times of clots containing increasing amounts of streptokinase (Lederle-Varidase). Three-tenths of a ml. of 0·6 per cent human fibrinogen was mixed with 0·1 ml. containing 1 N.I.H. unit of thrombin (15 sec. clotting time) and 0·2 ml. of a streptokinase dilution; the clots were incubated at 45° C. and observed for lysis. It is essential to employ a series of streptokinase concentrations since full activation of plasminogen by streptokinase occurs only at an optimal ratio of the two substances: too high concentrations of streptokinase depress plasminogen activation[4,5]. Streptokinase does not directly activate bovine plasminogen present in the thrombin; however, by interacting with traces of human plasminogen[6] or 'proactivator'[7] in the human fibrinogen, streptokinase will form 'activator' which now will convert bovine plasminogen to plasmin. Table 2 shows lysis-times of clots made as described above with (*a*) untreated bovine thrombin, and (*b*) thrombin treated with 0·5 *M* 2-mercaptoethylamine, both thrombins in the 1 : 70 dilution which gave a 15-sec. clotting time. The results indicate a very high degree of inactivation of the plasminogen present as a contaminant in the thrombin preparation. Samples of human plasmin assayed on clots formed with thrombin purified with alcohol or treated with 2-mercaptoethylamine gave identical results indicating that treatment with the latter did not interfere with the lysis of clots by plasmin.

Occasional reactivation of the plasminogen, presumably by re-oxidation of the disulphide bonds on

Table 1. Comparison of the Clotting Times of Thrombin* before and after Treatment with 0·5 *M* 2-Mercaptoethylamine

Dilution of thrombin	Thrombin untreated	Thrombin treated with 2-mercaptoethylamine
1 : 10	3 sec.	5 sec.
1 : 20	5 "	6 "
1 : 50	11 "	10 "
1 : 70	14 "	14 "
1 : 100	26 "	25 "

* 'Topical Thrombin' (Parke, Davis and Co.) 10,000 N.I.H. units dissolved in 10 ml. of 0·05 *M* imidazole buffer, *p*H 7·2, used as starting solution. Further dilutions made with the same buffer.

Table 2. Comparison of the Lysis Times of Clots made with Human Fibrinogen and 1 N.I.H. Unit of Thrombin before and after Treatment with 0·5 *M* 2-Mercaptoethylamine

Streptokinase units per clot	Thrombin untreated	Thrombin treated with 2-mercaptoethylamine
None	no lysis	no lysis*
0·1	45 min.	no lysis*
1	29 ,,	15 hr.
10	31 ,,	15 ,,
50	65 ,,	no lysis*
100	72 ,,	no lysis*

* Clots observed for 3 days.

storage, was prevented by treatment of the preparation with 0·1 *M* iodoacetamide after the dialysis has reduced the concentration of mercaptoethylamine to approximately 0·01 *M* as estimated by the nitroprusside test. The excess iodoacetamide was then dialysed out. This procedure, while it sometimes slightly decreases clotting activity, was found to give increased stability.

Inhibition of plasmin by sulphydryl compounds has been reported[8,9]. In experiments to be described elsewhere we have found that 2-mercaptoethylamine inactivates not only plasmin but also plasminogen. The inactivating effect of 2-mercaptoethylamine on plasminogen is probably due to its reducing property, rather than to the formation of an unactivable complex, since the effect persists after removal of the reducing agent. The most likely explanation of this effect is the splitting of one or more disulphide bonds essential for the activity of the plasminogen molecule. Structural distortion following reduction of disulphide bonds has been observed for several other proteins[10]. Since some of the disulphide bonds of thrombin also were probably reduced, as suggested by the increased *p*H sensitivity of these preparations, we conclude that the readily reducible disulphide bonds are not critical for the maintenance of the functional structure of the thrombin molecule.

Both treated and untreated thrombin tested at a high concentration (10,000 N.I.H. units per ml.), in the absence of streptokinase, lysed the purified clot in 5 hr. Mercaptoethylamine treatment, therefore, did not interfere with the fibrinolytic activity of thrombin itself[11,12].

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Blood Trehalose and Fat-Body Glycogen in the Silkworm, *Bombyx mori*

Since the report by Wyatt and Kalf[1] that trehalose is the principal carbohydrate in silkworm blood, attention has been paid to the biosynthetic process of this disaccharide in insects. Treherne[2] showed that trehalose labelled with carbon-14 is synthesized from labelled glucose, fructose and mannose in the locust, *Schistocerca*, and Candy and Kilby[3] demonstrated with this insect that the fat-body participates in the biosynthesis of trehalose, where adenosine triphosphate and uridine diphosphate glucose are required as cofactors for the enzymatic reactions involved. However, there are no publications dealing extensively with the correlation between *in vivo* formation of trehalose and various carbohydrates ingested by insects. As for the biosynthesis of glycogen, such a correlation has been elucidated, to some extent, with the fat body and other tissues of insects[4].

I have recently determined total blood carbohydrates and fat-body glycogen in the silkworm, *Bombyx mori*. The phenol–sulphuric acid method[5] was employed for the assay of total carbohydrates and glycogen, which was precipitated in alcohol in the presence of added sodium sulphate after digesting lyophilized fat-body in alkaline solution[6]. The same method was used for assaying trehalose, which was extracted from the trehalose spot developed on the filter paper by means of the solvent: *n*-butanol 4, acetic acid 1, and water 1. Trehalase obtained from silkworm midgut was also successfully used for trehalose determination.

When starved, fat-body glycogen decreased rather quickly, to a 60 per cent level of the original in 6 hr., and to a 15 per cent level in 12 hr. This level was maintained during prolonged starvation until the larva started feeding again. The original glycogen-level of the fat-body was resumed in several hours by feeding. Rather a different figure was obtained with total carbohydrates of the blood, the level of which did not decrease during early starvation period, but began to descend around the time when the amount of fat-body glycogen had reached the minimum level. The decrease was gradual so far as 49 hr. starvation. Food intake at the end of starvation resulted in a rapid increase in blood carbohydrates. The adminis-

Table 1

	Carboyhdrates	Increase in fat-body glycogen	Increase in blood trehalose
Pentose	Arabinose	−	−
	Xylose	±	±
	Ribose	−	−
	Rhamnose	−	−
Hexose	Glucose	+ + +	+
	Fructose	+ + +	+
	Mannose	±	±
	Galactose	±	±
	Sorbose	−	−
Disaccharide	Sucrose	+ + +	+
	Maltose	+ + +	+
	Cellobiose	+ + +	+
	Melibiose	+	+
	Trehalose	+	+
	Lactose	+	+
Trisaccharide	Melezitose	+ +	+
	Raffinose	+ +	+
Polysaccharide	Dextrin	±	−
	Starch	+	±
	Glycogen	−	−
	Inulin	−	−
Glycoside	Methyl-α-glucoside	−	−
	Methyl-α-mannoside	−	−
Sugar alcohol	Sorbitol	+ + +	+
	Mannitol	−	±
	Inositol	−	−
	Dulcitol	−	−
	Erythritol	−	−

tration of some carbohydrates to the starved larvæ also resulted in an increase in blood trehalose. Thus, the increase in both trehalose and glycogen after carbohydrate administration (20 mgm. per larva) in the form of a solution was determined by the use of larvæ which had been starved for 49 hr. The rates of increase were different with different carbohydrates, as shown in Table 1.

The most efficient sugars for the increase in fat-body glycogen were glucose, fructose, sucrose, maltose, cellobiose and sorbitol. Melezitose and raffinose were effective, but not so efficient as glucose. Melibiose, trehalose, lactose and starch were intermediate. Xylose, mannose, galactose, and dextrin occasionally resulted in some increase. No effect was obtained with sorbose, arabinose, ribose, rhamnose, methyl-α-glucoside, methyl-α-mannoside, mannitol, inositol, dulcitol, erythritol, glycogen or inulin.

Glucose was the most efficient also for increase in blood trehalose, and the list of carbohydrates falling into this group includes fructose, sucrose, maltose, cellobiose, melibiose, trehalose, lactose, melezitose, raffinose and sorbitol. Xylose, mannose, galactose, mannitol and starch seemed to be slightly effective, but not consistently. Sorbose administration resulted in an accumulation of sorbose in the blood, instead of an increase in trehalose. A similar result was obtained with arabinose, ribose, rhamnose, methyl-α-glucoside and methyl-α-mannoside. Inositol, dulcitol, erythritol, dextrin, glycogen and inulin were not efficient.

It is apparent from those results that the carbohydrates causing an increase in blood trehalose are generally also effective for that in fat-body glycogen. There is a good agreement in the kinds of carbohydrates between nutritive values[7] and efficiency of the increase in either trehalose or glycogen. Thus, the method determining the rate of increase in either compound by the use of starved larvæ is considered to be useful for the evaluation of nutritive values of carbohydrates.

Further details of this work will be published elsewhere.

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Reduced Folic Acid Analogues as Antimetabolites

Hydrogenation of the folic acid analogues aminopterin (4-aminopteroylglutamic acid) and amethopterin (N^{10}-methyl-4-aminopteroylglutamic acid) is reported to result in diminished antibacterial activity[1,2] against *Streptococcus faecalis R* (A.T.C.C. 8043) and *Pediococcus cerevisiae* (A.T.C.C. 8081). In view of the lability of tetrahydrofolic acid it seemed desirable to repeat these experiments taking precautions to avoid destruction of the reduced analogues; hence they were added to the assay medium in a potassium ascorbate solution after autoclaving[3]. The ascorbate did not greatly affect the growth of either organism. The media employed were similar to those described[4,5].

Table 1. Antibacterial Action of Folic Acid Analogues

Analogue	Amount of inhibitor producing a 50 per cent decrease in turbidity (mμgm./ml.)[a]	
	S. faecalis R[b]	*P. cerevisiae*[c]
Aminopterin[d]	0·35	> 100
Tetrahydroaminopterin	0·09	2·5
N^{10}-Formylaminopterin[e]	0·40	> 100
N^{10}-Formyltetrahydroaminopterin	0·03	2·0
Amethopterin[f]	0·13	50
Tetrahydroamethopterin	0·02	20

[a] Ascorbate concentration in all tubes, 0·6 mgm./ml.
[b] Contained 0·5 mμgm. folic acid/ml.
[c] Contained 0·4 mμgm. of the calcium salt of *dl*-N^5-formyltetrahydrofolic acid/ml.
[d] Commercial preparation hydrogenated without further purification.
[e] Commercial preparation partially purified by adsorption on 'Dowex-1'-chloride and elution with hydrochloric acid prior to formylation and hydrogenation.
[f] Lederle 85 per cent pure obtained from Dr. V. Oliverio, National Cancer Institute.

The hydrogenated analogues were synthesized by the procedure described for the preparation of tetrahydrofolic acid[6], and each product yielded four atoms of hydrogen per mole on iodine titration. They were dissolved in 1 *M* potassium phosphate, *p*H 7·8, in serum bottles filled with hydrogen. All dilutions were carried out with a sterile ascorbate solution (6 mgm./ml., *p*H 6·0).

As shown in Table 1, the reduced compounds are considerably more inhibitory in every instance than the corresponding unreduced analogue. The inhibitory action of aminopterin and N^{10}-formylaminopterin for *P. cerevisiae* is increased more than 50-fold by hydrogenation. Since reduced folic acid is required for the growth of this organism, the reduced analogues are most likely inhibiting at the tetrahydro level, rather than the reduction of folic acid to tetrahydrofolic acid where the unreduced antifolic compounds are known to act[7,8]. This suggestion is supported by the fact that with *S. faecalis R* it is more difficult to reverse, with folic acid, the inhibition caused by the reduced analogues than that caused by the unreduced analogues (Table 2). However, the inhibition caused by the reduced compounds is readily reversed by N^5-formyltetrahydrofolic acid ('Leucovorin') in both organisms excepting the case of the tetrahydroamethopterin inhibition of *S. faecalis R*. It therefore appears as if the enzyme sites involved do not in general bind the tetrahydro analogues tightly.

In the only cell-free enzyme system so far tested, the tetrahydrofolic acid-dependent methionine syn-

Table 2. Reversal of the Antibacterial Action of Folic Acid Analogues

Analogue	Amount of reversing agent required to restore half-maximum turbidity (mμgm./ml.)[d]		
	S. faecalis R[a]		*P. cerevisiae*[b]
	Folic acid	'Leucovorin'[c]	'Leucovorin'[c]
Aminopterin	5·8	2·0	—
Tetrahydroaminopterin	35·0	1·0	80
N^{10}-Formylaminopterin	1·5	0·8	—
N^{10}-Formyltetrahydroaminopterin	> 80·0	1·6	80
Amethopterin	50·0	4·4	—
Tetrahydroamethopterin	> 80·0	15·0	20

[a] Contained 1·0 mμgm. analogue/ml.
[b] Contained 0·1 μgm. analogue/ml.
[c] Calcium salt of *dl*-N^5-formyltetrahydrofolic acid.
[d] Ascorbate, as in Table 1.

thesis in *Escherichia coli* extracts[5] was inhibited 50 per cent by $2{\cdot}5 \times 10^{-4}$ *M* tetrahydroaminopterin or ^{10}-formyltetrahydroaminopterin, one-third the concentration of the tetrahydrofolic acid present. The unreduced analogues in each case were not inhibitory under the same conditions. In contrast, tetrahydroamethopterin was no more inhibitory than amethopterin. Both compounds yielded only 20 per cent inhibition when equimolar with tetrahydrofolic acid ($7{\cdot}5 \times 10^{-4}$ *M*).

These findings suggest that the hydrogenated analogues may be effective therapeutic agents and that they may also be useful in enzyme studies.

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Biotin in the Synthesis of Fatty Acid and Cholesterol by Mammalian Liver

Wakil, Titchener and Gibson[1] have shown that the formation of malonate from acetate and carbon dioxide is an early step in the synthesis of fatty acids in cell-free preparations of pigeon liver. They have also shown that biotin is necessary for this reaction, since it is inhibited by avidin, a specific inhibitor of biotin. By observing the effect of avidin, we have been able to show that, in cell-free preparations of rat liver, biotin is also required for the synthesis from acetate of fatty acids, but is not required for synthesis of cholesterol. It is thus possible to inhibit one biosynthetic pathway without influencing the other.

Homogenates of rat liver were prepared in ice-cold buffer by Bucher and McGarrahan's method[2], and were centrifuged for 20 min. at 2,000*g* to remove nuclei and cell debris, and then for 25 min. at 10,000*g* to remove mitochondria. The supernatant from the second centrifugation, containing the lighter subcellular particles, was transferred to flasks for incubation at 37° C. with air as the gas phase. Each flask received 2·5 ml. of supernatant, 20 μmoles of carbon-14-acetate as precursor of fatty acids and cholesterol, and the additions shown in Table 1. After incubation for 2 hr., the contents of the flasks were hydrolysed with sodium hydroxide. Fatty acids as soaps and cholesterol as digitonide were then isolated from the hydrolysate and assayed for radioactivity by methods previously described[3]. Table 1 shows the results of a typical experiment. The specific activity of the fatty acids from the flasks containing avidin without biotin was less than 10 per cent of that in the controls. Addition of biotin restored the synthesis of fatty acids almost to the control-level. Avidin had no significant effect on the specific activity of the cholesterol isolated at the end of the incubation.

Table 1. Each flask received 2·5 ml. of liver supernatant fraction and 1·0 ml. of buffer (magnesium chloride, 0·005 *M*; nicotinamide, 0·03 *M*; potassium phosphate, 0·1 *M*, *p*H 7·4) containing reduced coenzyme I, 1·0 μmole; coenzyme II, 0·5 μmole; glutathione, 30 μmoles; adenosine triphosphate, 17·5 μmoles; glucose-6-phosphate, 10 μmoles; potassium bicarbonate, 35 μmoles; potassium malonate, 25 μmoles; coenzyme A, 0·1 μmole; sodium 1-carbon-14-acetate, 20 μmoles/5 μc.

Additions	Specific activity (counts/min. at infinite thickness)	
	Fatty acids	Cholesterol
None	24,600	1,860
Biotin, 10 μgm.	32,500	1,860
Avidin (equivalent to 2 μgm. biotin)	1,910	1,690
Avidin + biotin	23,300	1,735

The avidin used in these experiments was kindly supplied by Miss H. Hellig, who prepared it by Woolley and Longsworth's method[4].

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PHYSIOLOGY

Effect of Season on the Follicle Stimulating Hormone and Luteinizing Hormone Potency of Sheep Anterior Pituitary Glands

It has been shown in the ewe that the 'total gonadotrophin' hormone content of the pituitary remains about the same during the breeding and during the non-breeding season (Warwick[1]). Kammlade *et al.*[2] noticed a rather higher total gonadotrophin hormone-level in the pituitary of the ewe during the non-breeding than during the breeding season. Lamond *et al.*[3] found no seasonal variation.

We[4] have carried out work on the change in levels of follicle stimulating hormone and luteinizing hormone (interstitial-cell stimulating hormone) activity of the pituitary glands of sheep collected at precise stages of the œstrous cycle. In this communication a comparison is made between some of these values and values obtained during the non-breeding season.

The anterior lobes of pituitary glands collected from Welsh mountain ewes kept under controlled conditions have been studied. The seasonal distribution for the collection of these glands in given in Table 1.

Table 1

Reproductive state	Time of year when samples were collected
36 hr.*	7.11.56 – 12.12.56
10 day*	20.11.56 – 14.12.56
Anœstrus	25.7.57 – 28.7.57

* From onset of œstrus.

All glands were excised, the posterior lobe removed, and the anterior lobe trimmed, weighed and frozen over solid carbon dioxide within 15 min. of death. Electrical stunning was not used on any of these animals. The individual glands were then freeze-dried, ground to a fine powder and stored in a desiccator over calcium chloride. The number of glands for each group was 9. For the follicle stimulating hormone determination, aliquots of the samples to be assayed were pooled, homogenized in ice-cold saline and assayed using a slight modification of the Steelman and Pohley[5] augmentation technique. A $2 \times 2 \times 1$ slope-ratio design with five rats per dose was employed and the assays were analysed by the method of Finney[6,7]. The follicle stimulating hormone potencies of the pituitaries assayed are expressed in terms of a purified ovine follicle stimulating hormone standard[8]. Estimates of the luteinizing hormone potency of the glands were carried out using the method of Parlow[9]. For these assays pituitary homogenates were extracted with ice-cold saline. A 2×2 or 2×1 design with five rats per dose was employed and the assays were analysed by the method of Gaddum[10]. The luteinizing hormone potency of the pituitaries assayed is expressed in terms of a purified ovine luteinizing hormone standard[11]. The results obtained are given in Table 2. It should be noted that in some cases the fiducial limits are rather wide.

Table 2

Stage of cycle (from onset of œstrus)	Potency (% of standard preparation)	Fiducial limits (95%)	Type of assay
Follicle-stimulating hormone values			
36 hr.	0·071	0·042–0·102	$2 \times 2 + 1$
10 day	0·143	0·111–0·183	$2 \times 2 + 1$
Anœstrus	0·180	0·145–0·228	$2 \times 2 + 1$
Luteinizing hormone values			
36 hr.	0·472	0·301–0·776	2×1
10 day	1·535	0·748–9·040	2×1
Anœstrus	1·051	0·471–6·682	2×2

From the above values it would appear that the follicle stimulating hormone and luteinizing hormone potency of the pituitary gland during the anœstrous state is of a similar order to that found during the breeding season. The value for follicle stimulating hormone is as high as the maximal value found during the pre-ovulatory phase of the cycle while the value for luteinizing hormone is similar to the mean value for the cycle.

During the non-breeding season the ovary is far from inactive in the ewe. The ovaries from the non-breeding ewes in the present work have as many large (4 mm. diam. or greater) follicles as, and a greater number of small (2–3 diam.) follicles than, the ovaries of ewes during the œstrous cycle. The size of the largest follicle in the ovaries of the ewes during the non-breeding season is similar to that for ewes during the breeding season excluding a 20 hr. pre-ovulatory period. No recent corpora lutea were present in the ovaries of the ewes during the non-breeding season, indicating a lack of ovulation during this time. If the maintenance of the large follicles found in ovaries of ewes during the non-breeding season is under gonadotrophin control then a constant or cyclic release of gonadotrophin from the pituitary should occur at this time. There must also be a constant production to maintain the levels of follicle stimulating hormone and luteinizing hormone which we have found in the pituitary. Kammlade *et al.*[2] suggested that the lack of ovulation during the breeding season was due to a deficiency of luteinizing hormone in the pituitary. The values reported here for the potency of luteinizing hormone of the pituitaries would indicate that the pituitary of the non-breeding ewe is not devoid of the hormone. The lack of ovulation observed during the non-breeding season may be due to a lack of a sudden substantial release of luteinizing hormone and follicle stimulating hormone from the pituitary such as we[4] have found to occur during the ovulatory phase of the œstrous cycle, rather than to a lack of these hormones in the gland.

A full account of this investigation is being published elsewhere.

We are indebted to Messrs. Armour Laboratories for the samples of purified follicle stimulating hormone and luteinizing hormone, to Messrs. Parke, Davis for chorionic gonadotrophin and to Messrs. Boots for pregnant mares' serum gonadotrophin.

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Uptake of Small Resin Particles (1–5μ Diameter) by the Alimentary Canal of the Calf

The intact skin and mucous membranes of the healthy body appear to present an impenetrable barrier to the entrance of particulate material, but yet bacteria are able to enter. How they do so is unknown. The tonsil is thought to be a vulnerable site: *Mycobacterium tuberculosis* has been shown to enter here[1], and even carmine granules dusted on to the tonsils before surgical removal penetrated very rapidly into the connective tissues[2]. The wall of the small intestine is also permeable to bacteria; coliform organisms[3], *M. tuberculosis*[1] and *Salmonellae*[4] have been shown to pass through the apparently intact mucous membrane and spread to the mesenteric lymph nodes, the blood, liver and spleen. More recently, at this Station, we have found that *Mycobacterium johnei* is also capable of rapid and extensive invasion of the body after the administration of an oral dose of culture to calves (Payne, J. M., and Rankin, J. D., unpublished work).

It was decided to see if inert particles would be taken up in the same way as bacteria. Previous experiments of this kind, using inert particles such as carmine[5], graphite[6] and polymethyl methacrylate spheres[7], to imitate bacteria have had limited or no

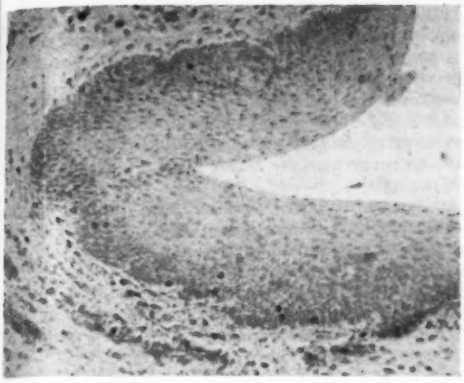

Fig. 1. Radioactive particles in the wall of the pharynx. Hæmatoxylin stain. (× 80)

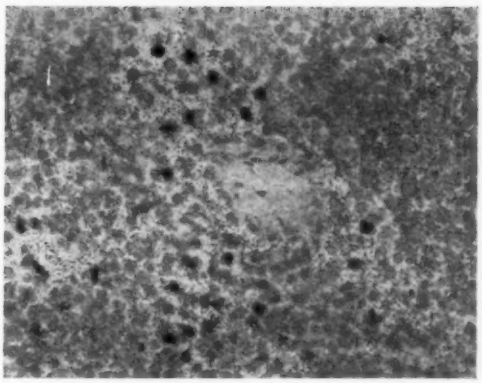

Fig. 2. Radioactive particles in cells lining a sinus in a lymph node. Hæmatoxylin stain. (× 360)

success, for even when fed in large amounts they only passed through to a limited extent or not at all. In view of the difficulty of estimating small quantities of these substances, we decided to use radioactive particles, which had the advantages of being easily detected and counted in body tissues.

Radioactive resin particles were prepared by one of us (B. J. M.). A stock suspension of these was made by grinding resin beads in a vibratory ball mill, followed by gravity sedimentation, centrifugation to eliminate large particles and finally filtration, with repeated washing, through a series of sintered glass filters so as to collect particles known to be between 1 and 5μ in diameter. Gold-198 was then adsorbed and reduced on to a sample of the final preparation. By this means a product could be obtained containing 50 mgm. resin particles of size 1–5μ, firmly labelled with 40 mc. of gold-198, having a total count of $1{\cdot}7 \times 10^{10}$ counts/min. The stability of the gold on the particles was checked by re-filtration through a No. 5 sintered glass filter. Only trace amounts of radioactivity came through into the filtrate, indicating firm labelling. This check was important, for in a preliminary experiment some loss of gold from the particles was observed.

Table 1

Lymph nodes	Counts/min./10 gm.	Tissue and fluids	Counts/min./10 gm.
Parotid	763	Tonsil	1,627
Mandibular	941	Pharynx	301
Suprapharyngeal	189	Lung	700
Mediastinal	2,483	Liver	189
Bronchial	794	Kidney	293
Hepatic	118	Spleen	242
Mesenteric	182	Muscle	56
Ileocæcal	361	Testis	246
Iliac	176	Blood	70
Precrural	1,718	Bile	25
Prescapular	2,052		
Popliteal	1,576		

The dose of particles was mixed with milk and fed to a calf six weeks old which was killed 72 hr. later and a variety of tissue samples removed and their radioactivity estimated in a well-type γ-scintillation counter. All counts were corrected for radioactive decay to the time of dosing. In addition, tissue sections were rapidly prepared by routine histological methods and covered with autoradiographic stripping film so that the precise localization of radioactivity could be determined. The results are shown in Table 1.

Radioactivity was relatively high in the tonsil and certain lymph nodes but low in the blood, muscle and bile. This result is, as would be expected if the particles were absorbed through the tonsil and alimentary canal, followed by distribution in the reticulo-endothelial system of the body. Confirmation of this was obtained by autoradiography, in which the localization of apparently individual particles could be traced in the tissues by the presence of tiny areas of dense blackening in the stripping film. By this means particles could be detected in the wall of the tonsil, pharynx (Fig. 1) and small intestine. Within the body they appeared to be associated with cells of the reticulo-endothelial system, particularly in lymph nodes (Fig. 2) and the spleen.

We conclude from these experiments that small numbers of particles of 1–5μ diameter can penetrate through the mucous membrane of the tonsil, pharynx and small intestine and be carried throughout the body, in apparently the same manner as bacteria. We suggest that the use of radioactive particles of this kind may prove to be a sensitive method for the study of various problems of infectious disease and possibly also in work on nutrition and nutritional allergy. Further details and results of experiments now in progress will be reported elsewhere.

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Influence of Tranquillizing Drugs on Phagocytosis *in vivo*

Phagocytosis of invading micro-organisms has long been known to play a major part in the defence of the mammalian organism against infectious disease. In view of the widespread use and potent pharmacological activity of the tranquillizing drugs, it is not surprising that administration of such compounds has occasionally been associated with alterations in normal resistance to infection[1,2], and that such altered clinical responses have been linked to changes in the phagocytic activity of the reticulo-endothelial system[3].

Vinegar and Berger[4] have recently reported that, in high doses, chlorpromazine, hydroxyzine and reserpine slowed the clearance of carbon particles from the blood of mice, but did not do so in low doses. Carbon particle clearance was not altered by meprobamate in either high or low doses. On the other hand, at the low dose employed by Vinegar and Berger, Ludány[5,6] found marked inhibition by chlorpromazine of the phagocytic activity of leucocytes from peripheral blood of dogs and rabbits.

In an effort to approximate more closely natural conditions than either carbon clearance or *in vitro* phagocytosis, an *in vivo* bacterial study was undertaken. Large male Wistar rats in groups of four were injected intraperitoneally with tranquillizing agents suspended in 5 per cent acacia solution. Three doses, 8 hr. apart, were administered to the experimental animals while control animals received 5 per cent acacia solution on the same schedule. All food was withheld during the experiment since it has been reported that phagocytic activity is enhanced by starvation[7].

Eight hours after the last injection of tranquillizer or acacia solution, each animal received 20 ml. intraperitoneally of broth culture of *Staphylococcus aureus* filtered through sterile glass wool and diluted with sterile saline to give approximately 50 per cent transmittance on a Coleman 'Junior' spectrophotometer. After 3 hr., animals were killed by decapitation and the peritoneal cavity opened. Duplicate slides of the contents were prepared, stained and counted for phagocytic activity by the method of Kuna *et al.*[7].

Table 1. Effect of Pretreatment with Tranquillizers on the Phagocytic Activity of Rat Leucocytes

Drug	Dose (mgm./kgm.)	Percentage of cells showing phagocytosis	Mean (per cent ± *S.E.*)	Significance of difference from control (*t* test)
5 per cent acacia (control)	—	32 38 24 31	31 ± 3	—
Chlorpromazine	25	63 71 86 70	73 ± 8	$P < 0.01$
Reserpine	1	20 17 27 25	22 ± 2	$P < 0.05$
Meprobamate	200	40 63 64 30	49 ± 8	$P > 0.05$
Hydroxyzine	25	30 31 22 36	30 ± 3	$P > 0.7$

Table 1 shows the effects of the four test agents on phagocytic activity as compared to acacia controls in a single run. It is apparent that pretreatment of rats with tranquillizing drugs resulted in marked alterations in phagocytic activity, with reserpine significantly depressing phagocytosis, chlorpromazine significantly enhancing phagocytosis, and hydroxyzine and meprobamate being largely without effect in this regard.

Analysis of variance[8] of the data obtained confirms the fact that the variation in phagocytic activity following drug treatment does not represent variation within a single population.

Repetition of the experiment at later dates gave comparable results, and the control values obtained by us are in agreement with reported values for untreated, starved rats[7].

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Influence of the Surface of the Small Intestine on Enzymatic Hydrolysis of Starch by Enzymes

The classical conception of digestion as being the sum of processes occurring in the chyme under the influence of hydrolytic enzymes fails to explain many important physiological and clinical phenomena.

In previous papers[1,2] it was shown that cavital (chyme) digestion is probably not the sole or main mechanism underlying the hydrolysis of food. It was suggested that the surface of the intestine presents a living porous reactor (the pores being formed by microvilli), on the surface of which intensive hydrolysis occurs on account of enzymes (self-contained and adsorbed) on the external side of the cellular membrane. (The presence of microvilli was first demonstrated by Granger and Baker[3] and later confirmed by many authors.)

The enzymes form a rather strong layer, which possibly has an ultrastructure analogous to what is known about enzymes on the membrane of mitochondria.

This is of great importance and suggests an enzymatic conveyer ensuring continued splitting followed by absorption of the food.

The system of processes occurring on the surface of the intestine we have designated as membrane or contact digestion[1].

Previous experiments have revealed that hydrolysis of larger molecules occurs mainly under the influence of dissolved enzymes while intermediate products are better split on the intestine surface. Thus, membrane digestion seems to be the intermediate link between cavital hydrolysis and absorption. For the latter it is the main source of providing substances for resorption[2].

The comparison of amylolytic hydrolysis *in vitro* and in the intestine has shown that the considerably greater speed of starch digestion in the organism is due to the operation of membrane hydrolysis and the interaction of the latter with hydrolysis in the chyme. These results were confirmed by model tests *in vitro*. It was established that the addition of a piece of living intestine to a pancreatic amylase solution results in a sharp increase in the speed of starch hydrolysis, which considerably exceeds the sum of the amylolytic activities of the solution and the intestine taken separately. It was further established that not only living intestine but also that first treated with trichloracetic acid and then washed, that is, an intestine without any enzymatic activity of its own, considerably increases the activity of pancreatic amylase. A special analysis has shown that a certain amount of dissolved enzymes is adsorbed on the surface of the intestine, and thus a system is created in the test tube in which interaction of cavital and membrane hydrolysis occurs[1,2].

Judging by the increase of amylase activity following the addition of mucosa treated with trichloracetic acid, membrane digestion is practically absent in the œsophagus, is only slightly marked in the large intestine and the stomach, and is well marked in the small intestine. Taking into consideration that membrane digestion should be preceded by cavital digestion and the former is closely related with absorption, such a topography will not seem casual.

Yet the question of what groups of animals are characterized by membrane digestion remained uncertain as former investigations were conducted on albino rats only.

The present work presents an investigation of the ability of pieces of mucosa taken from the small intestine of various animals to increase the activity of pancreatic amylase *in vitro*. Pieces of mucosa (1–1·5 sq. cm.) from the upper portions of the small intestine were taken from albino rats, albino mice, rabbits, cats, pigeons and frogs. The pieces were quickly taken from narcotized animals, washed in cooled Ringer's solution and then placed into a 5 per cent solution of trichloracetic acid. The concluding stage of treatment consisted of continuous and repeated washing in Ringer's solution at the temperature of 2–4° C. (Methods of preparing trichloracetic acid models are described in greater detail elsewhere[1]. The amylolytic activity was determined colorimetrically by the reduction of starch iodine staining Smith-Roy's method, somewhat modified.) The concentrations of the pancreatic crystalline amylase were so selected that the starch hydrolysis was 30–50 per cent in 30 min. at 38° C. and *p*H 7·2.

Special tests proved the absence of substances activating the amylase in the trichloracetic acid intestine models.

As shown in Fig. 1, the mucosa of the small intestine of a pigeon, albino mice, rabbit and a cat possesses the ability to increase amylase activity *in vitro*, approximately to the same degree as the well-investigated small intestine mucosa of the rat. I consider

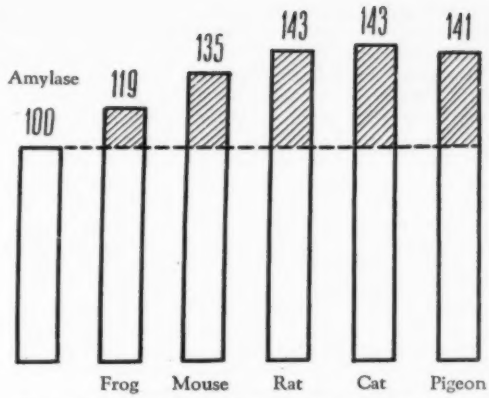

Fig. 1. Influence of pieces of mucosa from the small intestine on starch hydrolysis with amylase in various animals. Increase of activity is shown by the hatched part of the column above the background line. The activity of amylase solution is taken as 100 per cent; the trichloracetic acid intestine models possess no amylolytic activity of their own

this effect (with the reservations quite natural in such cases) to be a manifestation of membrane activity. The above experiments suggest the assumption that membrane digestion is inherent to all higher animals having various types of food ingestion. It is interesting that the same phenomenon, though to a lesser degree, is caused by the mucosa of the small intestine of the frog in which the typical villi are absent. Yet in Amphibia microvilli have been described which we are inclined to consider the structural basis of membrane digestion. If only this assumption is correct, membrane digestion must occur in the great majority of animal groups, for microvilli have been described even in roundworms[4].

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HÆMATOLOGY

Genetic Aspects of Two Different Minor Hæmoglobin Components found in Cord Blood Samples of Negro Babies

During work on the hæmoglobin types in the cord blood of Negro babies 70 samples were investigated by starch-gel electrophoresis[1]. The following results were obtained: 52 cases with HbA and HbF; 10 cases with HbA, HbF, and HbS; 3 cases with HbA, HbF and HbC; 3 cases with HbA, HbF, HbS, and a fast-moving component I; 1 case with HbA, HbF, and a fast-moving component II and 1 case with HbA, HbF, HbS, and a fast-moving component II. Using the carboxymethylcellulose chromatographic method[2,3], component I was found to be present for 3·2, 1·4 and 2·9 per cent respectively and component II for 5·6 and 6·5 per cent respectively. Work on the isolated fast-moving hæmoglobin fractions (prepared

by *CMC* chromatography) have shown that the two minor fractions were entirely different in structure[4–6]. These differences may be summarized as follows: (*a*) component I was slightly more resistant towards alkali than HbA, while the denaturation-rate of component II was intermediate between those of HbA and of HbF; (*b*) the ultra-violet spectrum of component I was similar to that of HbA, while the ultra-violet spectrum of component II was of the type found for HbF; (*c*) 'Amberlite *IRC*-50' chromatography of the globins prepared from the two hæmoglobin fractions at *p*H 1·9 with a urea gradient from 2 to 8 *M* revealed the presence of only β-polypeptide chains in component I and only γ-chains in component II; (*d*) sickle-cell hæmoglobin was formed as hybrid from a mixture of component I and normal HbA, and fœtal hæmoglobin from a mixture of component II and HbA. It seems, therefore, that component I (called 'Augusta I') consisted entirely of the β-chains of HbS, while com onent II is identical with Bart's hæmoglobin in being composed solely of the γ-chains of fœtal hæmoglobin.

Hæmoglobin studies of family members were possible for one family with the 'Augusta I' component and for two families with the Bart's hæmoglobin. The hæmoglobin samples were studied by starch-gel electrophoresis[1], by *CMC* chromatography[2,3] and by an alkali denaturation procedure[3]. In the 'Augusta I' family, the father and his sister, the mother, seven children (including the new-born baby) were available. In the blood of the new-born child HbA, HbF, 3·2 per cent of 'Augusta I', 0·8 per cent of HbS were demonstrable; the percentage of the fast-moving component being decreased to 2·1 per cent and the amount of HbS increased to 1·9 per cent[5], in a period of 45 days. Small amounts of an Hb-H-like component were also demonstrable in the cord blood sample. The mother and one of the children were completely normal; no increased percentages of HbF were detectable, while the HbA_2 component was present for 1·9 and 2·1 per cent. The father, his sister and three other children were heterozygous for HbS. Next to this abnormal hæmoglobin, however, minute amounts (about 0·1 per cent) of a fast-moving fraction were demonstrable. This fraction possessed electrophoretic properties similar to those of HbH (or β_4^A). It seems, therefore, that these family members were also 'heterozygous for the α-chain deficiency'[7], resulting in the formation of very small amounts of HbH. The two other children were heterozygous for the sickle cell hæmoglobin; no fast-moving fraction was demonstrable in the hæmolysates of their red blood cells. These results indicate that two genetically determined hæmoglobin abnormalities were present in this family[5], both being inherited independently.

In the first family with Bart's hæmoglobin, blood samples obtained from the father, the mother, and seven children (including the new-born baby) were available. The amount of Bart's hæmoglobin in the blood of the new-born baby (as determined by *CMC* chromatography) was 5·6 per cent, fœtal hæmoglobin was present for 60·7 per cent, while a small amount of HbA_2 (0·5 per cent) also was demonstrable. The hæmoglobins of both parents and of the six other children showed no abnormalities; the percentages of fœtal hæmoglobin were less than 1 per cent, while the amounts of HbA_2 ranged between 1·9 and 2·3 per cent.

In the second case, the percentage of Bart's hæmoglobin was 6·5 per cent, while next to HbA, HbF (68 per cent), small amounts of HbS (4·7 per cent) and of HbA_2 (0·2 per cent) were present. Blood samples of both parents were available for study. The hæmoglobin of the mother was completely normal (HbF less than 1 per cent, HbA_2: 1·9 per cent), while the father had the sickle-cell trait (HbS : 34·5 per cent, HbA_2: 2·1 per cent, HbF less than 1 per cent). No other abnormalities were demonstrable.

The results of these family studies are of some special genetic interest since in the first family an abnormality in the production of the adult hæmoglobin types HbA and HbS is present during fœtal and adult life, while in the other two families an abnormal fœtal hæmoglobin is formed during fœtal life without any visible alteration in the pattern of adult hæmoglobin. It is likely that all three new-born babies suffered from a 'heterozygous α-chain deficiency', resulting, however, in the formation of entirely different 'α-chain lacking' abnormal hæmoglobin types. These findings suggest a qualitative difference in the α-chain deficiency, to be explained by a suppression of the formation of the adult α-chain in members of the first family and of the fœtal α-chain in members of the other two families. Therefore my results offer evidence that the production of adult and fœtal α-chains is controlled independently by two α-genes, which, however, must be almost identical in fine detail since the α-chains of HbA and of HbF do not show structural differences by the finger-printing technique, or by hybridization[8–10]. The adult α-chain deficiency seems to be more pronounced during fœtal life, but is still visible after birth by the production of small amounts of HbH. The 'fœtal α-chain deficiency' is confined to the synthesis of fœtal hæmoglobin; thus, no abnormalities are detectable in adult life.

The possibility exists that a heterozygosity for both adult and fœtal α-chain deficiencies is present in one individual and results in the presence of an abnormal adult hæmoglobin (as 'Augusta I') and of the abnormal fœtal hæmoglobin (Bart's hæmoglobin) in the same cord-blood sample. Evidence for the existence of this double heterozygosity was obtained by demonstrating two fast-moving minor hæmoglobin components in the blood of a new-born baby next to HbA, HbF and small amounts of HbC. The fastest-moving minor fraction was electrophoretically similar to Bart's hæmoglobin, while the slowest-moving minor fraction ('Augusta II') was probably composed solely of the abnormal β-chain of HbC. Unfortunately, examination of the hæmoglobins of family members was not possible.

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The Diego Factor in Japanese

It is already known that the study of blood groups and types and their distribution and heredity are of great significance in anthropology.

Since the Diego blood group was originally reported[1] in 1954 the investigation of this blood group and its incidence in different races of various countries have been reported by several authors. At present, the Diego factor has been found in the blood of South American Indians, and mixed bloods in Venezuela, Brazil, North American Indians, and Chinese and Japanese residents in Canada and Venezuela. No Diego factor was discovered in Caucasians and Negroes; therefore it seems that the Diego factor is an anthropological characteristic of Indians and Mongoloids.

The first investigation of the Diego factor in Japanese in Japan was carried out by Furuhata, Yokoyama and Kuniyuki[2] in Tokyo, and they found 2 (2·3 per cent) Diego positive (Di(a+)) bloods in 88 samples. The anti-Diego serum was kindly supplied by Dr. Philip Levine, Ortho Research Foundation, Raritan, New Jersey, for this investigation. The serum showed activity and clear-cut results by indirect Coombs test.

We received another antiserum through the courtesy of Dr. Miguel Layrisse, Banco de Sangre, Caracas, Venezuela, for the second study. This time we used serum to test the blood of people in Tokyo and in Kumamoto located in the southern part of Japan. Tests were made by the indirect Coombs technique and the results were 6 (4·1 per cent) of Di(a+) bloods in 146 random samples in Tokyo and 17 (7·5 per cent) in 227 unrelated people in Kumamoto.

Masaki and Furukawa[3] reported the frequency of the Diego factor as 16 (3·2 per cent) in 500 Japanese living in the Gumma area. They used anti-Diego serum obtained from Dr. James F. Mohn, Buffalo University, Buffalo, New York.

The incidence of the Diego factor in Japanese from our investigation is approximately 3–4 per cent in Tokyo and is a little lower than the 8 in 65 cases (12·3 per cent) in Venezuela reported by Layrisse and Arends[4] and the 6 in 77 cases (7·8 per cent) in Canada reported by Lewis, Ayukawa, Chown and Levine[5]. However, the incidence of the Diego factor was found to be about 7·5 per cent in the southern part of Japan. Moreover, Dr. Paul J. Schmidt (personal communication), Blood Bank, National Institutes of Health, United States, found an incidence of nearly 10 per cent of the Diego factor in samples from Fukuoka located on the same island as Kumamoto. One of us (N. U., personal communication) examined samples in Kumamoto more recently and found 8·7 per cent Diego-positive bloods.

Table 1. Frequency of Diego Factor in Japanese

Authors	Tested No.	Diego factor	
		Pos. (%)	Neg. (%)
Layrisse and Arends (1956)	65	8 (12·3)	67 (87·7)
Lewis, Ayukawa, Chown and Levine (1956)	77	6 (7·8)	71 (92·2)
Furuhata, Yokoyama and Kuniyuki (1957)	88	2 (2·3)	86 (97·7)
Masaki and Furukawa (1959)	500	16 (3·2)	484 (96·8)
Yokoyama, Murakata and Ueno (1960)	146*	6 (4·1)	50 (95·9)
	227†	17 (7·5)	210 (92·5)

* Tested in Tokyo. † Tested in Kumamoto. The number includes 5 (6·7 per cent) Diego positive of 74 random samples previously cited by Layrisse, M., *Amer. J. Phys. Anthrop.*, **16**(2), 173 (1958).

From our present results, there appears to be a lower incidence of the Diego factor in northern Japan, with the incidence gradually increasing and reaching the highest incidence in Japanese in the southern part of Japan. This frequency suggests that the Diego factor may be an Asiatic characteristic as are factors for group B, Rh_o, kk and Gm^{a} [6].

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RADIOBIOLOGY

Alkoxyglycerols as Growth-stimulating Substances

In connexion with experiments on irradiated rats, it has been shown that the alkoxyglycerols or their esters prohibit to a certain extent the decrease both of megakaryocytes and of nucleated cells in the bone marrow, a decrease which usually occurs after irradiation[1]. When radiomimetic substances (for example, leukeran and myleran) are given to rats, a diminution of the above types of cells is equally observed. This diminution is also partly prevented by alkoxyglycerols.

It has now been found that the alkoxyglycerols have an effect not only on the bone marrow, but they also influence the growth of the rats. In general, normal, non-irradiated rats increase in weight more rapidly than irradiated rats. However, when irradiated rats were prophylactically treated with alkoxyglycerols the increase in weight was higher than for the irradiated control group, which received no treatment. The normal non-irradiated group showed an increase of 1·7 gm. a day per animal, the prophylactically treated group 1·3 gm. and the control group 1·1 gm.

When the alkoxyglycerols were found to promote the growth of rats, it seemed desirable to try their effect on *Lactobacilli*, which have been used as test organisms for many growth-stimulating substances. Twelve strains of *Lactobacilli* were tested. Pure synthetic media were used as substrates. The growth was determined turbidimetrically. The best results were found for *Lactobacillus lactis* (Fig. 1). The cultures were transferred to two sets of tubes, one control set with synthetic substrates and one with substrates to which had been added selachyl alcohol (an unsaturated alkoxyglycerol). A very striking effect is observed at a concentration of 20γ per ml. of alkoxyglycerols, but even at a concentration of 2γ per ml. a clearly observable effect is obtained. (I am indebted to Dr. Lennart Enebo for the performance of these experiments.)

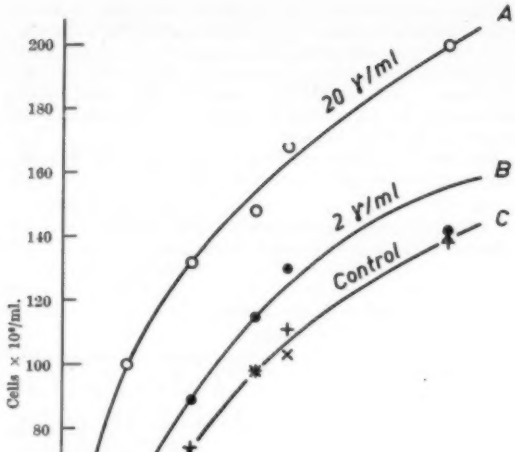

Fig. 1. Effect of selachylalcohol on the growth of *Lactobacillus lactis*. *A*, Synthetic substrate + 20γ selachylalcohol per ml.; *B*, synthetic substrate + 2γ selachylalcohol per ml.; *C*, control, synthetic substrate only

The observation that alkoxyglycerols stimulate growth is of interest since these substances are found in the blood-forming tissues (cf. ref. 2). From experiments on rats (*loc. cit.*) it was found that the alkoxyglycerols had an effect on both megakaryocytes and nucleated cells (from which derive several types of cells in the peripheral blood). The blood-stimulating factor vitamin B_{12} has an effect only on the red cells.

Very little is known of the formation of alkoxyglycerols in the body and of their presence in different foods. The effect of alkoxyglycerols in nutrition would be an interesting subject for further investigations.

A more detailed report of these experiments will be published elsewhere.

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An Apparent Difference in the Renal Clearance of Calcium-45 and Calcium-40 in Rats

In investigating the comparative excretion of calcium-45 and strontium-89 in rats we have encountered evidence of unexpected variability in the renal clearance of calcium-45 and calcium-40. This finding may be significant in metabolic work employing radiocalcium as a tracer.

Weanling female Sprague–Dawley rats (group I) were injected intraperitoneally with 1·0 ml. of a solution containing carrier-free strontium-89, calcium-45, and 0·05 mgm. of calcium. Using methods previously described[1] urine was collected at the end of 30 min. and 60 min., and analysed for strontium-89, calcium-45 and total calcium. One hour after injection, blood was obtained from each rat by cardiac aspiration, and the concentration of strontium-89 and calcium-45 in the plasma was determined. A comparable group of rats (group II) was denied food for 12 hr. and the experiment repeated. The results are presented in Tables 1 and 2.

Table 1. Concentration of Strontium-89 and Calcium-45 in Plasma

Group	No. of rats	Percentage of dose/ml.		
		Strontium-89	Calcium-45	Sr-89/Ca-45
I	6	0·50 ± 0·03*	0·49 ± 0·02	1·01 ± 0·02
II	8	0·56 ± 0·02	0·56 ± 0·20	0·99 ± 0·01

* *S.E.*

Calcium-45 and strontium-89 occurred in slightly higher concentrations in the plasma of group II rats, yet these animals excreted considerably less of each during both collection periods. From the strontium-89/calcium-45 ratios in urine and in plasma it is further apparent that the renal clearance of calcium-45 was decreased more than that of strontium-89. This finding agrees with a previous report from this laboratory[1] which showed a progressive decrease with time in the renal clearance of calcium-45, relative to strontium-89, following the intraperitoneal injection of these isotopes.

Despite the markedly different excretion of labelled calcium, both groups of rats excreted equivalent amounts of total calcium. The reason for this is not apparent. It is unlikely that the short period of food denial would affect the total serum calcium. There is also no reason for believing that the renal clearance-rates of calcium-45 and calcium-40 differ intrinsically. While a slower absorption of the injected calcium-45 could account for its diminished excretion by group II rats, the results of a separate experiment would serve to rule out this possibility. Here it was found that the plasma of similarly starved rats contained a higher percentage of calcium-45 30 min. after injection than did that of non-starved controls, namely, 1·20 per cent and 0·89 per cent respectively. (Corresponding 1-hr. values were

Table 2. Urinary Excretion of Strontium-89, Calcium-45 and Calcium

Group	No. of rats	Period (min.)	Percentage of dose excreted			
			Strontium-89	Calcium-45	Sr-89/Ca-45	Calcium excreted (mgm.)
I	6	0–30	3·15 ± 0·60*	0·81 ± 0·26	7·2 ± 2·9	1·09 ± 0·07
II	8		0·31 ± 0·06	0·02 ± 0·001	13·0 ± 1·6	1·12 ± 0·09
I	6	30–60	1·31 ± 0·36	0·32 ± 0·14	5·4 ± 0·9	0·87 ± 0·05
II	8		0·17 ± 0·03	0·03 ± 0·008	6·2 ± 0·8	0·84 ± 0·03

* *S.E.*

0·52 per cent and 0·46 per cent.) An explanation for the disproportionate excretion of calcium-45 and total calcium under these conditions may possibly be found in a different partitioning of the injected label among the body calcium pools.

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Uptake of Phosphorus-32 by the Roots of *Lolium perenne*

PREVIOUS work on the ability of various portions of the root to supply the shoot with minerals has been carried out using the seminal roots of cereals grown in nutrient solution when the plants were a few days old. Weibe and Kramer[1] considered that in barley the most active zone of absorption and translocation of minerals was situated some distance behind the tip. The results of Lundegårdh[2], also working on barley, indicated that most nitrate was translocated from the basal portion of the root, that is, that nearest the shoot.

Young seminal roots grown in nutrient solution may not be directly comparable with older nodal roots. The present experiments were conducted with plants of *Lolium perenne* a few weeks old which had been grown in soil and possessed several nodal roots. Just before the experimental treatments the roots were washed free from soil. All plants the roots of which showed signs of decay were rejected.

The absorption and translocation of phosphorus were studied using phosphorus-32. This was received carrier-free in dilute hydrochloric acid, which was added as required to the Hoagland solution. The plants were allowed to absorb the phosphorus-32 from this solution for 6 hr. The amount in the shoot tissue after this period was estimated by drying the material, digesting it with concentrated sulphuric acid and then measuring the activity of the resulting liquid, after making it up to a known volume.

In the first series of experiments, the ability of one portion of a root to absorb and translocate phosphorus-32 was studied. A portion of 12 mm. of one root of each plant was isolated by passing the root through a cellulose test-tube which had two holes opposite each other in its side. The junctions between the root and the test-tube were made watertight with simple ointment. Radioactive Hoagland solution (2 ml. with an activity of 5 μc.) was put in the test-tube while the rest of the root system was bathed in normal Hoagland solution.

The results of one experiment with white unbranched nodal roots 10–12 cm. long are presented in Table 1. The basal region absorbed most phosphorus-32 which was translocated to the shoot. Similar results were obtained in other experiments.

Table 1. UPTAKE OF PHOSPHORUS-32 AND ITS TRANSLOCATION TO THE SHOOT FROM THREE PORTIONS OF THE ROOT

Portion	Base	Middle	Tip
Shoot weight (gm.)	0·0515±0·0092	0·0614±0·0068	0·0548±0·0080
Counts per min.	2,855±697	605±105	462±231
Counts per unit weight	6·3±1·5	1·1±0·3	1·0±0·4
No. of plants measured	6	6	6

Table 2. EFFECT OF CUTTING OFF THE ROOTS AT 2 CM. FROM THE BASE OF THE SHOOT ON THE UPTAKE OF PHOSPHORUS-32 AND ITS TRANSLOCATION TO THE SHOOT

	Uncut	Cut	*S.E.*
Shoot weight (gm.)	0·0705	0·0728	0·0041
Counts per min.	1,447	1,055	140
Counts per unit weight	2·03	1·46	0·19
Total root weight (gm.)	0·0362	0·0337	0·0037
Weight of root cut off (gm.)	—	0·0259	—
No. of plants measured	12	12	—

The value of the basal portion of the root was confirmed by an experiment in which plants with intact root systems were compared with plants the roots of which had been cut off 2 cm. from the base of the shoot. The roots of both series of plants were immersed in Hoagland solution to which phosphorus-32 had been added to give it an activity of 100 μc. per litre. Although the 'cut' plants had 77 per cent of their roots removed, the amount of phosphorus-32 reaching the shoots was only reduced by 28 per cent.

These experiments strongly suggest that it is from the basal portions of the roots that the shoot receives most of its phosphorus. The top few inches of the soil would therefore appear to be most important for the mineral nutrition of the plant. Boggie *et al.*[3] found that grasses absorbed more phosphorus-32 from the upper 10 cm. of the soil than from greater depths.

According to Stout and Hoagland[4] the phosphorus-32 would have to reach the xylem before it could be distributed throughout the plant. It would seem, therefore, that the thickened endodermis found in this region of the root[5] was not an effective barrier to the inward movement of ions, as suggested by Weibe and Kramer[1]. Alternatively, there may have been considerable downward movement of phosphorus-32 in the cortex either to the younger regions of the root where the endodermis was not thickened, as suggested by Soper[5], or upwards to the junction of the root and shoot. If the thickened endodermis was an effective barrier, the plants with 'cut' roots in the second experiment must have absorbed the phosphorus-32 through the exposed xylem on the cut surface.

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BIOPHYSICS

Physico-chemical Properties of Purified Cytochrome *a*

THE enzymatic and spectral properties of purified cytochrome *a* isolated from animal tissues in soluble form have been studied by many investigators. However, a number of questions remain unanswered as to its physico-chemical properties. In previous

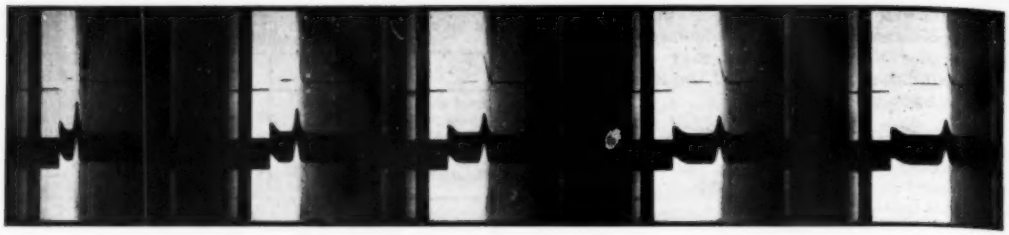

Fig. 1. Sedimentation pattern of cytochrome *a* in 0·1 *M* phosphate buffer containing 0·25 per cent 'Emasol 1130', *p*H 7·4. The direction of sedimentation is from left to right. The numbers under the photographs indicate the time in minutes after the rotor attained full speed (51,000 r.p.m.). The average rotor temperature was 16·4° and the protein concentration 0·54 per cent. (The small peak shown on the left may be due to detergent)

communications[1,2] we have reported that a synthetic non-ionic detergent effectively reactivated purified cytochrome *a* while an ionic detergent was inhibitory. To study cytochrome *a* in an enzymatically active state in the presence of a non-ionic detergent, we have carried out various sedimentation experiments by ultracentrifuge. The highly purified cytochrome *a* preparation used in these experiments was prepared from ox heart muscle as described in the previous communication[2]. Cytochrome *a* was dialysed against 0·1 *M* phosphate buffer containing 0·25 per cent 'Emasol 1130' at *p*H 7·4 for 12 hr. The sedimentation pattern of cytochrome *a* in the presence of the non-ionic detergent, 'Emasol 1130', showed a single homogeneous and symmetric monodisperse component, as seen in Fig. 1, whereas in the presence of the ionic detergent, sodium cholate, the protein behaved as a polydisperse, aggregated system.

The sedimentation constant was determined at different protein concentrations and found to vary slightly with the protein concentration. A corrected value of $S_{20,w} = 21·9$ Svedberg units was obtained. Diffusion was measured on a preparation which had been dialysed overnight against the solvent system used for the sedimentation experiments. From results at different protein concentrations, the mean value was calculated as $D_{20,w} = 3·58 \times 10^{-7}$ cm.^{2} sec.$^{-1}$. The partial specific volume was found to be 0·72 ml. gm.$^{-1}$. Combining these values in the familiar Svedberg equation, a molecular weight of 530,000 was obtained. The minimum molecular weight calculated from the hæm, iron and copper contents was 100,000. Based on these results it is concluded that cytochrome *a* has about five molecules of hæm and five atoms of copper per molecule of protein. However, Criddle and Bock[4] recently succeeded in obtaining monomeric cytochrome *a* by treating a preparation with sodium dodecyl sulphate and estimated the molecular weight to be 72,000. Therefore, assuming that cytochrome *a* contains only one hæm and one copper atom per molecule, it would be expected that some kind of polymerization takes place and a monodisperse pentamer of cytochrome *a* exists in the non-ionic detergent solution. To test this possibility ultracentrifuge experiments are now in progress in our laboratory. The frictional ratio f/f_0 was calculated to be 1·12. If the molecule is an unhydrated rotational ellipsoid, the axial ratio would thus be about 3 : 1. Further detailed results on the physico-chemical properties of cytochrome *a* will be reported elsewhere.

We wish to express many thanks to Messrs. K. Kakiuchi and T. Takagi of Isemura Laboratory of this University for helpful advice on the physico-chemical measurements.

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A Potentiometric Method for Observation of Osmotic Behaviour of Prawns

In the course of work on osmotic regulation of estuarine invertebrates, it was found that cryoscopic and vapour-pressure methods do not yield reproducible results in a tropical place such as Madras. The potentiometric method outlined here, which is based on the predominance of chloride ions in the blood of animals and in sea water, facilitates a continuous picture of changes in the concentration of the blood of an animal in a given medium and helps us to measure the amount of energy involved.

A simple concentration cell containing 2 per cent sodium chloride solution immersed in 0·5–4 per cent sodium chloride solutions registers potential differences varying from 0·5 to 30 mV., the e.m.f. developed being proportionate to the ratio of the difference in concentrations When such a cell was used with sea-water of 16·2 per mille sodium chloride within the cell and sea-water varying from 4·05 to 32·4 per mille outside, it was found that in spite of the occurrence of other ions besides sodium and chloride, the silver–silver chloride electrodes used indicated potential differences varying in the same way as before. At the laboratory temperature (30° C.) the e.m.f. will be $60 \times \frac{87}{109} \times \log_{10} \frac{c_i}{c_o}$, allowing for the conductances of sodium and chloride ions and also the fact that the concentration and junction potentials are involved.

Table 1

Concentrations of outside media (per mille)		4·05	8·10	16·20	20·25	24·30	32·40
e.m.f. in mV.	Internal medium : sea water 16·2 per mille (observed)	27·24	13·68	0·30	4·86	8·12	13·56
	Internal medium : sea water 16·2 per mille (calculated)	28·40	14·38	0·00	4·65	8·34	14·38
	Internal medium : blood of prawn	23·60	11·70	3·01	0·31	2·10	7·60

Values calculated thus for the concentration differences are given in Table 1 along with values recorded by experiment. The e.m.f. increases by 14·5 mV. when the external concentration was doubled, as expected from the calculations.

In estimating the concentrations of the blood of the prawn in sea water of different salinities, a silver – silver chloride electrode is left in the container while the second electrode, shaped like a needle, is inserted in the heart of a live prawn, tied to a glass slide and left in the container. The results are given in Table 1. It will be seen that in spite of the blood having a protein concentration of 80 gm./litre[1], and is surrounded by different tissues as well as cuticular membrane, the e.m.f. values indicate the differences in concentration fairly closely. Since the e.m.f. recorded is lowest (0·31 mV.) when the prawn is left in sea-water of 20·25 per mille, the chloride concentration of the blood may be inferred to be the same as that of outside medium. It may be noted here that Panikkar and Viswanathan[2] found the chloride concentration of the blood of this prawn (*Metapenaeus monoceros*, Fab.) to be roughly 20 per mille, determined by the Volhard titration method as described by Wigglesworth. This method is now being used by one of us (O. R. R.) for work on osmotic behaviour of this prawn. Since the electrode is sharp and slender, the prawns can easily survive the injury in about eight out of ten cases even though they are used every day over a period of ten days.

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BIOLOGY

Different Types of Body Movement in the Hagfish, *Myxine glutinosa* L.

From the systematic and anatomical points of view, Myxinoids are well known[1–4]; but there are as yet only short notes about their living habits[2,4,5]. During the summer of 1959, I had the opportunity of examining living hagfishes at the Universitetets Biologiske Stasjon Drøbak in the Oslofjord, Norway. I observed about 200 hagfishes living in aquaria and also freshly captured specimens.

Observations on the habits of hagfishes will help in describing the life-history of these animals. I propose to report here particularly the movements of the animals when disturbed in their self-produced slime masses. There are to be distinguished complicated moving patterns of the body of the hagfishes, the result of which is the cleaning and liberation of the surface of the body from adherent slime. This cleaning seems to be of essential importance for a normally functioning respiratory stream from the nasopharyngeal duct to the gills.

The animals were captured by means of eel-pots furnished with fish as bait[5]. Most of the observations were made with hagfishes captured from a depth of about 50 metres in the Oslofjord near Drøbak, also some at the Biologiske Stasjon Espegrend near Bergen in western Norway. Freshly captured animals were put as quickly as possible into aquaria furnished with sea water of a medium temperature of about + 8° C. In the aquaria there was a continuous flow of new sea water taken from a depth of 30–50 metres.

Normally the hagfishes lie nearly the whole time at the bottom of the containers, most of them stretched out, some rolled up. Animals disturbed by touching with a stick move in lively fashion for some minutes but return shortly after the end of the stimulus to their usual motionless position. Hagfishes which have not been fed for some days become very active as soon as food is offered, for example, small herring, annelids, etc.

The hagfishes show normal locomotion movements comparable to those of eels, snakes or worms. The body of the animal makes sinuous swaying movements caused by alternate contractions and relaxations of opposite muscle segments of the body (compare Fig. 1). Usually the animals move forward with the head as the front pole. But often, especially if touching an obstacle, for example, the wall of the container or another animal, the movement may be reversed so that the tail end becomes the front pole of movement. Also under such conditions of backward swimming, snake-like swaying occurs. The plane of the swaying movement is often, but not always, horizontal. Locomotion is possible in all directions of space with the same accuracy. Sometimes a sort of rotation along the main body axis is observed, giving an impression comparable with driving a screw.

Fig. 1. Normal snake-like swimming movement in the hagfish

Excited animals—for example, after repeated stimulation by a stick, but also spontaneously in the case of animals disturbing one another by slime production (for example, in crowded containers)—show other types of movement besides the normal snake-like swaying. These special movements are in most cases connected with the liberation of the animals from slime masses covering their body surface.

Roughly two types of special movements can be distinguished: (1) The simpler one consists in a repeated rather narrow figure-of-eight movement of the body along the main body-axis. The general impression is similar to the usual snake-like type of locomotion, but the animal does not move forward or very little (Fig. 2). (2) The second type is more conspicuous and, I believe, is connected with a more intense excitement of the animals. At first the hagfish forms a loop by means of the end of its tail (Fig. 3). The loop remains close to the surface of the body and in the following stage of the movement the whole body is pulled through the loop, thus wiping away the slime from the surface of the body. The direction of this type of movement is from the tail towards the head. After passing the mouth and the nostril the loop opens again. The general impression of this type of movement is that of 'knotting' the body. The time necessary for one knotting movement is about 2–3 sec. Such knotting-movements are normally performed twenty times or more. After having finished with knotting, the animal swims off, moving then in the normal snake-like manner, and after some seconds or even minutes of swimming it comes to rest again in the usual manner at the bottom of the container.

Both types of special movements (figure-of-eight motion and knotting) are, according to my observations, always connected with intense excitement of the animals and are therefore to be regarded as 'excitement movements'. Intense excitement in most cases leads to the production of large amounts of slime. The types of movement described therefore serve to liberate the animals from self-produced slime, and also from slime produced by other members of the species or disturbing agents. Slime seems to be dangerous to the animals not only mechanically but also by the interruption of the water-stream from the nostril through the craniopharyngeal duct into the gills and thus causing cessation of 'breathing'. Before performing the special movements, the animals are often as it were exhausted; but after repeated 'knotting' and 'figure-of-eight motion' their bodies assume their normal motion.

The two types of special movements are performed in different positions of the body in relation to the bottom of the container, mostly of course in a general horizontal position.

It is obvious that these patterns of movement with their exact step-by-step decrease have a functional

Fig. 2. Typical appearance during figure-of-eight motion

Fig. 3. Knotting motion in *Myxine glutinosa*. Succeeding stages from left to right, schematic

background in a central nervous mechanism released by special stimuli.

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Transplantation of Mammary Glands

I wish to describe two methods I have used of transplanting whole mammary glands in goats. The work was undertaken so that mammary function could be studied in conscious undisturbed animals, by experimenting on one gland and comparing it with the control gland on the same animal. Anæsthesia reduces the rate of milk secretion, and it is not easy, for example, to make repeated flow measurements or arterial infusions in conscious goats because the main artery is difficult to exteriorize effectively. By transplanting one gland on to another site on the animal, the main artery and vein are made readily accessible. It is well known that mammary growth and milk secretion can take place after the nerves to the tissue have been removed, and it is generally

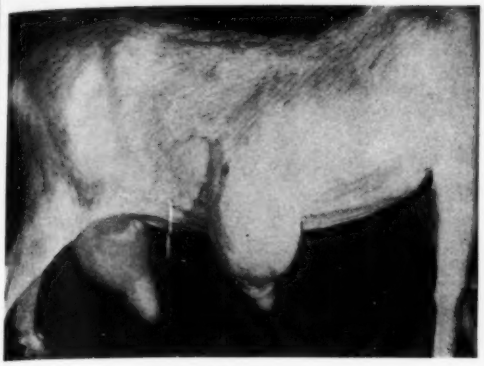

Fig. 1

believed that these processes are almost entirely controlled by hormones. Although Ribbert showed in 1898 [1] that small pieces of mammary tissue survive autotransplantation and show histological signs of normal growth and function, there have been no reports of the transplantation of whole glands nor of the quantitative measurements of growth and lactation of denervated tissue that are possible with the present methods.

The first results were unexpected since apparently successful transplants made in young kids failed to grow normally during pregnancy and yielded much less milk than the control glands in the normal situation. This was reminiscent of the long-term deficiencies following section of the spinal or sympathetic nerves to mammary tissue reported in some laboratories[2]. With modifications in technique, other transplanted glands have grown almost normally and the maximum yields have reached 70–90 per cent of the controls during lactation.

(1) *Transplantation in 2 or 3 stages.* By this method the gland was moved in stages to its new site (on the side or back) in such a way that it always retained a vascular link with the animal. It has been done in four virgin animals just before the first pregnancy, and in one animal at the end of the first lactation (Fig. 1). At the first operation one gland (half the udder) was separated from the body except for the skin at its front and the caudal superficial epigastric vessels ('milk' vein and artery) lying beneath this skin. These vessels were thus the only source of blood, and since they anastomose with the cranial superficial epigastric vessels emerging through the abdominal wall by the xiphoid, once they had enlarged, the result was a gland supplied by a readily accessible artery and vein each 25–30 cm. long. During this phase the gland was replaced in the normal position for 3 weeks and then again dissected out on this long pedicle right back to the xiphoid region. A piece of skin the size and shape of the gland was removed over the last rib and the gland was pulled into this space by tunnelling under the skin, and then stitched into place. Although it is almost certain that the entire nerve supply is cut by these two operations, in one goat the caudal superficial epigastric vessels were resected at a third operation three weeks later. In another the artery was exteriorized as a skin-covered loop which has remained patent for two years.

(2) *One stage transplant.* This has been done only in two goats, both in full lactation. In both cases one gland was removed entirely from its normal site and stitched on to the ventral surface of the neck (Fig. 2), the main artery and vein being joined to the carotid artery and the external jugular vein respectively. The control gland was pulled over to close the gap left in the inguinal region and now lies in the midline. In the first animal the caudal superficial epigastric vessels were joined to the omocervical vessels with polythene cannulæ within 16 min. and the main vessels were anastomosed within 100 min. to the carotid and jugular end-to-side by Phelan's trapdoor technique[3]. The carotid was tied above the join one month later. In the second animal the vessels were joined end-to-end in 36 min. using 1 cm. long stainless steel Payr cannulæ outside the mammary vessels and then stitched. This animal was then given one dose of heparin (1,000 U./kgm.). At both operations the main efferent lymph duct was cannulated and brought to the exterior. However, after the operation the lymph-flow slowed down markedly, probably associated with general anæsthesia[4], and to prevent clotting the cannulæ were removed. A defective lymph drainage may be why both transplants became more swollen than the controls. Whereas the milk yields of the controls recovered within 4 days, the transplants took 10 and 16 days and are still slightly œdematous 6 weeks after the operation. It should be possible to exteriorize the mammary vessels later.

Some observations made on all these goats may be mentioned. The 2-stage technique is easier to apply to virgin animals but, at best, glands transplanted in this way have given only 70 per cent of the expected milk yield. Furthermore, in one animal a decrease in yield was seen in the second lactation after the

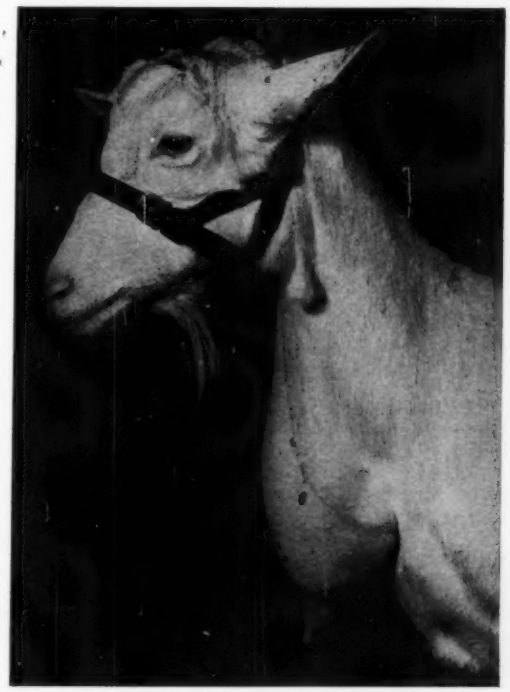

Fig. 2

transplantation and there is some evidence to suggest that the transplanted gland is affected by the presence or absence of the control. The newly acquired blood supply may well be inadequate. For this reason the one-stage technique would seem to be better and the milk yields of the two animals investigated are 75 and 90 per cent of that expected six weeks after the operation although the long-term results are not yet known. The operative technique could be improved by joining the blood vessels much more quickly and anastomosing the lymph duct to the lymphatics of the neck. In contrast to denervated glands or to those transplanted back into the normal site, when regeneration of sensory nerves has been fairly extensive during the course of pregnancy, the glands transplanted in two stages have remained completely anæsthetic for up to 2 years. In one animal tests made 2 years after transplantation showed that a small number of vasomotor nerves had entered the gland with a new artery that had grown in from the surrounding skin. In none of the animals has it been possible to initiate milk ejection or rumination by milking the transplanted teat. In cases where both glands were transplanted (1 animal) or the control gland was removed (2), lactation proceeded in the apparent absence of milk ejection. By injecting oxytocin intravenously (200 mu.) during each milking for a week at various stages of lactation it was possible to assess the importance of milk ejection; in this way it was found that 80–98 per cent of the milk may be obtained in the goat without it. Tverskoy[5] also noted that after complete denervation of both glands in one out of four goats the complete yield could be obtained without oxytocin.

Further work is in progress to try to decide to what extent the residual deficiencies noted may be due to impairment of the blood supply and lymph drainage.

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Inflation of the Neck Pouch of the Marabou Stork

The pouch of the marabou stork (*Leptoptilos crumeniferus* (Lesson), was described by Peters[1] and its nature investigated by Chapin[2]. Chapin concluded that the pouch was inflated by means of air from the left nostril being driven into a pair of thin-walled sacs in the neck.

Detailed dissection of specimens collected in the Nairobi area have revealed a complicated valvular mechanism in the left nasal organ, which directs air from the lungs into the neck sacs during exhalation. The left and right air sacs are inflated through the left nostril, air being directed by a series of valves behind and below the eye-ball and through a narrow passage over the pterygoid. This passage leads into the left air sac, which in turn communicates with a smaller sac on the right side of the neck. The left nasal organ is divided into two chambers, one leading directly from the external to the internal nares, and a second inflation chamber which leads from the external to the internal nares and also to the orbital passage by means of a valvular opening. The right nasal organ is similar but bears no opening to the orbital passage. Although this opening is closed, the passage and the pterygoid duct are both present.

Inflation of the neck pouch is accomplished in the following manner:

(1) *Inhalation*. Air is drawn into the lungs through the external nares, passing through either the nasal or the inflation chamber.

(2) *Exhalation*. When the air sac is to be inflated, the nasal chamber and the entrance to the inflation chamber are closed by means of a sphincter and a small constrictor muscle. When air is exhaled from the lungs it enters the ventral opening of the inflation chamber; whence it is directed by a concave valve through the posterior opening to the orbital passage and the air sac.

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Isolation of Transplantation Antigens from a Cultured Lymphoblast *L*-5178*Y*

Experiments designed to characterize the nature of the transplantation antigens have been greatly hampered by the difficulty of obtaining large amounts of a homogeneous starting material. We would like to report the successful isolation of transplantation antigens from a mammalian cell cultured *in vitro*.

Fischer[1] has shown that a lymphoblast, *L*-5178*Y*, that originated as a spontaneous leukæmia of thymic origin in a *DBA*/2 mouse can be grown in tissue culture. The cells do not stick to glass and can, therefore, be grown easily in suspension. In the medium reported by Fischer, yields of 2–3 gm. wet weight per litre are routinely achieved. The cells grow logarithmically *in vitro* and in the ascitic fluid of a *DBA*/2 mouse with an 11·4-hr. generation time, and can be transferred from one condition to the other with an adaptive lag in growth of less than 24 hr.

Adult C_3H mice immunized with 10,000 *L*-5178*Y* cells reject an intraperitoneal challenge of 2×10^6 *L*-5178*Y* cells, a dose that results in abundant growth of an ascitic tumour which is fatal to 23 per cent of the non-immunized controls. Such immunized C_3H mice also show an accelerated rejection of a graft of *DBA*/2 skin on the tail[4]. Test grafts of *C*57 *BL* skin on the tails of both immunized and non-immunized C_3H mice are rejected at the same time, 10–15 days, indicating that *L*-5178*Y* contains antigens specific for the *DBA*/2 strain.

The antigenic substances were isolated in a soluble form and attached to particulates from freshly harvested *L*-5178*Y* cells grown *in vitro*. The fractionation procedure is based on the methods used by Billingham, Brent and Medawar[2] with spleen and thymus cells. Cells were suspended in distilled water, and treated in a Raytheon 10 kc./sec. sonic oscillator

Table 1. Response of C_3H Mice to Immunization with Whole Cells and Cell-free Extracts of *L*-5178*Y* cultured *in vitro*

Antigenic material	Tumour challenge			Skin graft test		
	No. of mice tested	No. of survivors		No. of mice tested	Median survival time of grafts (days) *DBA*/2 C57*BL*	
		Showing ascitic growth	No detectable ascitic growth			
Control	30	22	1	5	13	12
Whole cells	10	0	10	6	6	—
Crude suspension	12	1*	11	4	7	—
Deoxyribonucleoprotein fraction	10	4*	6	6	7	12
Microsome fraction	10	2*	8	6	6	12
Soluble fraction	17	4*	13	7	6	13
*p*H 5 precipitate	10	2*	8	6	7	12

*The amount of ascitic growth found in these cases was always much less than that seen with controls tested at the same time.

in the cold for 1 min. at full power. After centrifuging for 10 min. at 3,000*g* to remove debris, the crude suspension was made 0·15 *M* in sodium chloride, and allowed to stand for 1 hr. in the cold. The pellet obtained after centrifuging for 10 min. at 5,000*g* consisted mainly of deoxyribonucleoprotein of the cells. The supernatant suspension was centrifuged for 1 hr. in the Spinco ultracentrifuge, model *L*, at 105,000*g*. This precipitate (microsomal fraction) and the soluble fraction were tested separately for antigenic activity. Each fraction was injected intraperitoneally into adult C_3H mice, so that each mouse received the extract from $1·5 \times 10^8$ cells. Five days later, the animals were challenged by each of the test procedures outlined above. Antigenic activity, that is, protection of C_3H mice from a challenge dose of *L*-5178*Y* and an accelerated rejection of *DBA*/2 skin (Table 1), was found in each of the three cell fractions to about an equal extent. Whether the antigenic material in each fraction is chemically different or whether equal amounts of the same antigen are distributed among the three fractions has not yet been elucidated. What is interesting to note is that we obtained an antigenic response to 1·5 mgm. of a soluble proteinaceous material in both tests. In several experiments the antigenic material was found to precipitate after 24 hr. in the cold when the soluble fraction was adjusted to *p*H 5. Castermans and Oth[3] have also reported the isolation of soluble antigenic material from the nuclei of mouse spleen and thymus cells.

A detailed report on the chemical properties of active fractions and the animal test procedures used will be presented at a later date.

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Gibberellin and Sex Differentiation of Flowering Plants

Though work on the influence of gibberellin on flowering of plants has been given much attention, up to now very little is known about the relation of gibberellin to sex differentiation of plants.

My experimental work was carried through with hemp (*Cannabis sativa* L., var. Rastislavická). Gibberellin was applied in aqueous solutions containing 5, 10, 25 and 100 p.p.m. The seeds were allowed to soak in the solutions 24 hr.: controls were in distilled water for the same time. The number of individuals in the different variations of the experiment ranged between 400 and 500, all variations of experiments being repeated twice. When examining the results I noted first of all the relation of gibberellin (*a*) to sex differentiation, (*b*) to growth of female and male individuals.

As to the relation of gibberellin to sex differentiation, an increasing percentage of female individuals following treatment with gibberellin was observed. The highest increase by 113·9 per cent (with regard to control plants) was registered with concentration 10 p.p.m.

Proportions of male and females plants $\left(x = \frac{a♂}{a♀}\right)$ after application of gibberellin were as follows:

control plants		$x = 1·07$
application of gibberellin at	5 p.p.m.	$x = 0·99$
	10 p.p.m.	$x = 0·82$
	25 p.p.m.	$x = 1·05$
	100 p.p.m.	$x = 1·04$

When comparing the average length of stems of female and male plants at the end of vegetative growth, different reaction of female and male plants to various concentrations could be traced, both with respect to stimulating (lower concentrations) and inhibitory (higher concentrations) effect. To a greater extent the stimulating effect was shown on female plants where the maximum of stem elongation was 107·8 per cent with a concentration of 5 p.p.m., the maximum elongation on male plants being 106·5 per cent at 10 p.p.m. (percentage given with regard to control plants). The inhibitory influence of higher concentrations (25 and 100 p.p.m.) was manifested in a greater measure with female plants. Induction of male sterility, however, was not observed; male plants showed normal development after application of gibberellin.

Because of the close relation of gibberellin to the synthesis of growth substances, as well as on grounds of observations made by Laibach and Kribben[1,2] and by others, according to which sex expression of female and male flowers depends on the concentration of growth substances in the organism, it may be presumed that the observed reduction of percentage and the higher growth inhibition in male plants and, on the contrary, the increase of percentage, the higher stimulatory and lower inhibitory effect in female plants, after application of various gibberellin concentrations is related in some way to the synthesis (to various concentrations) of growth substances in female and male plants. Their increased production (dissolution) from some precursor or their destruction after gibberellin treatment (especially at higher concentrations) is probably capable of influencing the biochemical processes of female and male plants considerably, which may find expression—as the observations mentioned show—not only in processes

of growth but also in the process of sex differentiation itself.

The gibberellin used in these experiments was obtained through the courtesy of the Kyowa Fermentation Industries, Tokyo, and Plant Protection, Ltd., Fernhurst Research Station, nr. Haslemere, Surrey, England.

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Effect of Growth Substances on the Osmotic Value of Pea Stem Sections

It has been shown that gibberellin does not increase the extensibility of the pea stem, while auxin does[1,2]. It thus remains to show whether gibberellin increases the osmotic concentration in cells, so as to increase water absorption. As to the effect of auxin on the osmotic concentration, conflicting results have been reported[3–5]. The present communication reports some experiments which reveal that gibberellin causes catatonosis while auxin causes anatonosis.

Pieces of stem, 10 mm. long, excised from an apical part of the third internode of etiolated Alaska pea seedlings were weighed and soaked in 20 mgm./l. solution of gibberellin (composed of A_1 4·5 per cent and A_3 90·0 per cent), 10 mgm./l. solution of indole-3-acetic acid or distilled water. After being floated on the solutions or water for 3 and 6 hr. at 25·5° C., the stem pieces were lightly blotted and packed in weighing tubes to prevent loss and condensation of moisture during weighing and freezing (at about −21° C.) to follow. After melting, tissue juice was obtained using a hand press. Freezing point of the juice was measured using a copper-constantan thermo-junction and potentiometer to the nearest 0·01° C.

Freezing-point depressions measured are presented in Table 1. The molar concentrations (w/v) of mannitol solution corresponding to them were determined by using a standard freezing-point curve prepared experimentally, and given in Table 1.

Let the water content of stem pieces be H and βH respectively before and after the treatment; and let the virtual amount of the 'solute' contributing to the osmotic concentration of tissue juice be S and αS respectively before and after the treatment. Then the osmotic concentration of press juice is $C_0 = S/H$ before the treatment, and $C_1 = \alpha S/\beta H$ after that. The solute change factor, α, is given by:

$$\alpha = \frac{C_1}{C_0}\beta \qquad (1)$$

C_0 and C_1 are represented by mannitol values. The water-absorption factor, β, is the ratio of the amounts of water (βH and H) which are determined by subtracting from the fresh weights the dry weight of the tissue. The last-named was estimated with the same stem pieces as used in the experiment.

The values of β in Table 1 show that pieces treated with indole-3-acetic acid absorbed more water than control, while those treated with gibberellin did not. On the other hand, the 'solute' contributing to the osmotic concentration of tissue decreased more conspicuously with indole-3-acetic acid and gibberellin treatments than water control. In these experiments, however, solutes may have diffused out of the tissue into treatment solutions. Hence, in order to determine if catatonosis is really caused by indole-3-acetic acid and gibberellin, stem pieces were kept in air of constant humidity, instead of being put in the solutions.

Air equilibrated with the saturated solutions of magnesium sulphate and potassium phosphate and with distilled water is considered to be of about 90 per cent (ref. 6), 96·8 per cent (ref. 7) and 100 per cent humidity, respectively, at 25° C. Pieces of stem were smeared at both ends with lanolin pastes of 1 per cent gibberellin and of 0·5 per cent indole-3-acetic acid, as well as with pure lanolin. They were incubated under slow circulation of the above-mentioned air for 3½ hr. at 25·5 ± 0·01° C. The fresh weight of these pieces (carrying the lanolin) were determined before and after the incubation, and press juice was obtained from the pieces after the

Table 1. Effects of gibberellin and indole-3-acetic acid solutions on freezing point depression of press juice, factor of water absorption, and factor of change in the amount of the osmotically effective solutes, when etiolated pea stem sections were floated on the solutions

	Freezing point depression (° C.)	Mannitol equivalent (M (w/v))	Water absorption factor (β)	Solute factor (α)
Before treatment	0·60	0·30	—	—
After treatment for 3 hr. with				
Water	0·48	0·24	1·14	0·91
Indole-3-acetic acid (10 mgm./l.)	0·38	0·19	1·36	0·86
Gibberellin (20 mgm./l.)	0·44	0·22	1·15	0·84
After treatment for 6 hr. with				
Water	0·44	0·22	1·18	0·87
Indole-3-acetic acid (10 mgm./l.)	0·29	0·15	1·57	0·78
Gibberellin (20 mgm./l.)	0·40	0·20	1·21	0·81

Table 2. Effect of lanolin pastes of gibberellin and indole-3-acetic acid on freezing point of press juice, factor of water absorption, and factor of change in the amount of the osmotically effective solutes, when etiolated pea stem sections smeared with the pastes were kept in air of constant humidity

	Freezing point depression (° C.)	Mannitol equivalent (M (w/v))	Water absorption factor* (β)	Solute factor (α)
Humidity (100 per cent)				
Control	0·60	0·30	0·996	1·00
Indole-3-acetic acid (0·5 per cent)	0·75	0·38	0·997	1·26
Gibberellin (1 per cent)	0·55	0·27	0·994	0·90
Humidity (96·8 per cent)				
Control	0·60	0·30	0·986	0·99
Indole-3-acetic acid (0·5 per cent)†	0·71	0·36	0·984	1·18
Gibberellin (1 per cent)	0·56	0·28	0·986	0·92
Humidity (90·0 per cent)				
Control	0·61	0·30	0·955	0·96
Indole-3-acetic acid (0·5 per cent)	0·76	0·38	0·957	1·21
Gibberellin (1 per cent)	0·56	0·28	0·947	0·88

* Water absorption factor less than unity represents loss of water.
† Elongation effect of indole-3-acetic acid was rather small in this experiment made in winter, as is usually the case.

parts smeared with lanolin were cut off. The results are shown in Table 2.

The moisture contained in the pieces decreased slightly even in air of 100 per cent humidity. This may perhaps be due to evaporation during the transfer to and from the container for incubation. The pieces treated with indole-3-acetic acid had high osmotic concentration, the solute increase being large. The gibberellin treatment, on the contrary, caused a decrease in the 'solute'. It is therefore suggested that anatonosis is caused by indole-3-acetic acid, and catatonosis by gibberellin.

It has often been shown that indole-3-acetic acid increases extensibility of the cell wall. Together with this, the fact that anatonosis is caused by indole-3-acetic acid presents a simple hypothesis which can explain the way this acid causes elongation. On the other hand, gibberellin rather causes catatonosis, and, as has been reported[1,2], makes pieces of stem elongate less under a stretching force. Hence the mechanism of action of gibberellin should be sought from some other point of view.

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Protein from Sugar Cane

SUGAR cane is a major crop in many areas of the world where there is also malnutrition, particularly a shortage of dietary protein, and a high birth-rate. It is important that these areas should exploit to the full any potential source of protein for human diets, either to be consumed directly or indirectly through animal feeding.

Pirie has canvassed widely for more attention to the potentialities of leaf protein extraction, but sugar cane leaves, in common with the leaves of all high-yielding crops, are very low in protein, containing only about 6 per cent on a dry-matter basis.

The basis of extracting leaf proteins is maceration, filtration and coagulation. This scheme is almost followed by sugar factories for preparing the cane juice prior to its concentration, the heat-coagulated protein being filtered off along with other solids to give filter cake which may be returned to the fields as a soil amendment or even dumped as waste.

The heat-coagulable nitrogen content of cane juice is very small, but nevertheless the quantities involved during a crushing season are high.

Mauritius, with an annual crop of around 550,000 tons of sugar, produces about 100,000 tons of filter cake with a nitrogen content of 1 per cent. In other words, about 6,000 tons of cane juice 'protein' are returned to the soil each year as a fertilizer in a country where protein-deficiency symptoms are common among the population.

In the laboratory it is simple to produce a precipitate containing up to 8 per cent nitrogen from cane juice; on a factory scale, however, the problem is more difficult.

Much work has been done in cane-producing countries on the separation of cane-wax, but no process has yet proved to be economical. As the coagulate formed in cane juice on heating carries much of the wax with it, a combination of protein and wax recovery may prove to be financially interesting.

Research work is in progress in Mauritius to study the amino-acid composition and nutritional values of the heat-coagulated precipitates and the technological aspects of their separation. The full results of this work will be published later.

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Microspectrophotometry of *Euglena* Chloroplast and Eyespot

A SIMPLIFIED microspectrophotometer developed in our laboratory was applied to obtain the *in vivo* absorption spectra for the chloroplast and eyespot of the algal flagellate *Euglena*. The optical and electrical characteristics of this instrument, which utilizes a Bausch and Lomb grating monochromator and a cadmium selenide photoconductive cell, have previously been reported[1].

Euglena gracilis Z strain (obtained originally from Dr. S. H. Hutner, Haskins Laboratory, New York) cultured in a chemically defined medium under continuous fluorescent light (300 ft.-candles) in a temperature-controlled room of 25° C. was used for this work. Samples of *Euglena* were taken, during their log-phase of growth, from the culture bottles, placed on a microscope slide with the edges of the cover-glass sealed with 'Vaseline' to prevent drying-out of the organism and pressure applied in order to immobilize them. No further preparation or staining was employed. The prepared *Euglena* slide was immediately placed on the microscope, a chloroplast or eyespot was selected and focused for scanning with the microspectrophotometer. The reference area was the suspending medium adjacent to the organism; the half-band width of the incident radiation was 4·6 mμ. The green chloroplasts measure about 1μ in diameter by about 10μ in length[2]. The orange-red eyespot is 1–2μ in diameter by 3μ in length; it consists of packed granules and is close to its effector, the flagellum[5,6].

In Fig. 1*a*, the absorption spectrum of an individual chloroplast over the wave-length range 250–340 mμ is illustrated. The results are presented as percentage absorption for intervals of 10 mμ. This spectrum is matched at 340 mμ with another chloroplast spectrum, Fig. 1*b*, extending to a wave-length of 700 mμ. Except for small changes in relative peak heights, the results were consistent from one chloroplast to another within the same organism as well as in different organisms under the same condition of time of growth and light intensity. These chloroplasts have two major absorption peaks, one in the region 430–435 mμ and the other in the region 675–680 mμ with minor peaks near 485, 585 and 620 mμ. The peaks were

previously noted from absorption spectra on cell suspensions of *Euglena*[3], although fine-structure details and the spectra from individual organelles were not obtainable. Among the absorption peaks listed above, the 485 mμ peak is known to be due to carotenoids, while the others are due mainly to chlorophyll *a*, which has peaks at 432, 480 and 665 mμ in methanol. In following the absorption peaks with culture age, changes in the 415–435 mμ as well as new peaks appearing at 685–690 mμ could be observed. In the ultra-violet region, an absorption peak in the region 265–270 mμ with lesser peaks at 290, 340 and 390 mμ would indicate that the chloroplasts contain proteins and lipids.

The eyespots of *Euglena* were investigated in the visible range, 400–700 mμ. The reference area in this case was the cell cytoplasm or the vacuole adjacent to the eyespot. The spectrum for an average eyespot is illustrated in Fig. 2. In contrast to the chloroplast spectra, the eyespot results exhibited appreciable variation in peak height from one eyespot to another. They show that the eyespot has a broad absorption maximum in the region 480–490 mμ, two sharper maxima at 510 and 530 mμ respectively, and lesser peaks at 430 and 630 mμ. An absorption peak at 450–460 mμ was noted for several eyespots, and is shown as a shoulder on the curve in Fig. 2. This spectrum is in qualitative agreement with that obtained by Gössel using a microbeam larger than the eyespot cross-section[4]. The eyespot therefore absorbs light throughout the entire visible range. The action spectra for *Euglena* phototaxis has peaks at 420 and 490 mμ, and for photokinesis (rate of swimming irrespective of direction) has peaks at 465 and 630 mμ[5]. The eyespot absorption spectrum therefore contains these same peaks as do the action spectra. The interpretation of Fig. 2 with respect to the identity of the pigments present in the eyespot is, however, difficult. Normal green strains of *Euglena gracilis* produce three main carotenoids: β-carotene, lutein and neoxanthin. In a non-polar solvent, carbon disulphide, the absorption range of these carotenoids is 450–510 mμ. These carotenoids could therefore account for a major proportion of the observed absorption. However, astaxanthin, which has a broad absorption band at around 500 mμ and has been found in red variants of *Euglena*, and not identified here, could be masked by the above carotenoids.

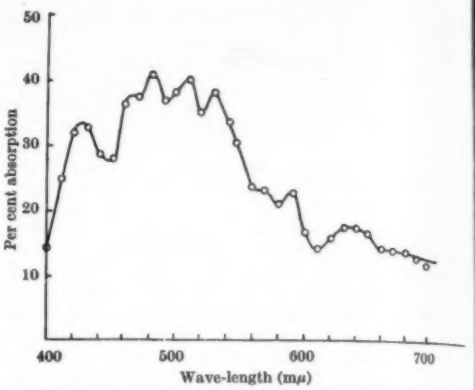

Fig. 2. *E. gracilis* eyespot average absorption spectrum

This work was aided in part by grants from the United States Public Health Service Institute of Neurological Diseases and Blindness (*B*–397 *C5*, *B*–1874–*A*) and the Stoner-Mudge Foundation. This work will be published in greater detail (*J. Protozool.*, 1961).

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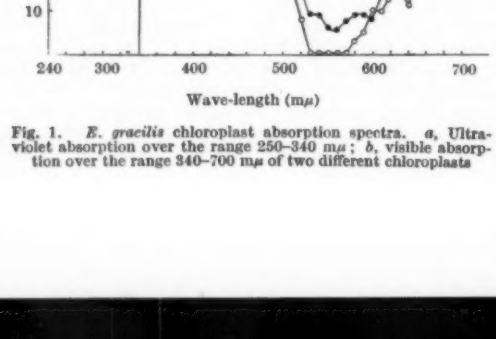

Fig. 1. *E. gracilis* chloroplast absorption spectra. *a*, Ultra-violet absorption over the range 250–340 mμ; *b*, visible absorption over the range 340–700 mμ of two different chloroplasts

MICROBIOLOGY

An Improved Method for photographing Superficial Microbial Colonies

The lack of contrast in transparent superficial microbial colonies causes some difficulties during photography. The following method promises good results provided some precautions are taken. Suppose it is intended to photograph transparent, colourless colonies on nutrient agar plates. The surface of the medium is flooded with indian ink diluted in distilled water (1 : 100). The ink contains a pigment, carbon black, having a particle size of 200 Å. (Pelikan Tusche No. 541, Günther and Wagner, Hanover). When the upper layer becomes coloured (about 2–3 min.), the surplus solution is sucked off. As a result, in the weakly coloured medium the colonies remain colourless and in a photograph the distinction is clearly visible (Fig. 1). The principle of this procedure is founded on the slower penetration of coarse-grained colloid into the colonies and may be considered as the macro-adaptation of Burri's indian ink 'negative staining' method[1]. One must be careful to work

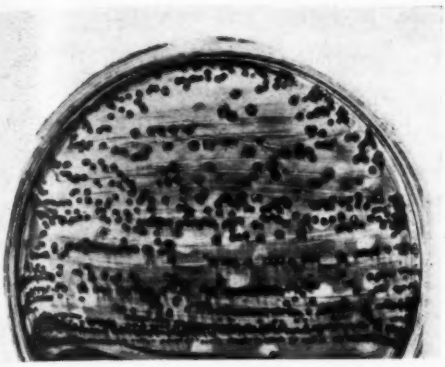

Fig. 1. Colourless and transparent colonies of *Salmonella paratyphi B* after treatment with diluted indian ink

quickly before the colonies become coloured and the dish should not be stirred because the colonies easily emulsify. The use of crossed 'Polaroid' filters or 'Polaroid *J*' film (Fisher Scientific) under and over the dish gives more contrasted pictures. As light source, an X-ray illuminator is suitable.

The advantages of the method seem to be at first in its application for the photographic documentation of colony counts, and for the visualization of small transparent colonies.

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CYTOLOGY

Determination of Sex in Granulopoietic Cells of Mice and Rats

WHILE examining Feulgen-stained sections of rat bone-marrow, it was observed that certain granulocyte precursors showed a body strongly resembling the sex chromatin described in other species[1] in that it occurred with much greater frequency in female than in male marrows.

In the initial work on the sex chromatin[1] it was not found possible to identify this body in rodents because of the coarse clumping of nuclear chromatin in the central nervous system, with which these studies were largely concerned. More recently, however, it has been shown that sex chromatin bodies may be demonstrated in those rodent tissues where the nuclear chromatin is sufficiently dispersed, for example, in liver cells[2,3], certain neurones[4], ameloblasts[5], amnion cells[3], cells in tissue culture[6] and a small proportion of peripheral blood leucocytes[7] in rats, and in neurones and liver cells in hamsters[3,8].

Accordingly, in view of the value a sex chromatin body could have as a marker in studies involving marrow transfer, the observations mentioned above were extended.

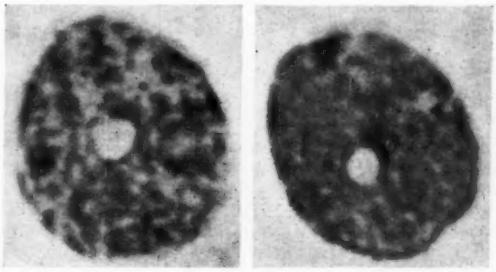
Colony-bred mice of *A2G*, *C57* and *AK* strains and rats of the *WAG* and *PVG* strains were used[9]. Femoral marrow was fixed in glacial acetic acid/ethanol (1:3) for 1 hr., re-suspended in water for 5–10 min., hydrolysed in *M* hydrochloric acid at 60° C. for 10 min., treated with Schiff's solution for 45 min. and transferred to chilled 45 per cent acetic acid. Permanent 'squash' preparations were made by the quick-freeze method[10].

A preliminary survey suggested that attention be concentrated on the cells classified by Harris and Burke[11] as 'typical metamyelocytes', large cells with ring- or doughnut-shaped nuclei, often with one or two nucleoli and showing, in Romanowsky-stained films, a few azurophil granules. In 'squash' preparations many of these cells show chromatin sufficiently dispersed to make observation of a sex chromatin body feasible. Only those cells were considered in which, apart from the bodies to be described, the chromatin was well dispersed, the nucleus showing a homogeneous lilac-stained background overlain by a finely condensed reticular or stippled pattern. Sometimes a few tiny splinter-like or nodular condensations were present, usually at the nuclear margins. Nuclei were excluded from consideration in which a sex chromatin body could not have been identified confidently had it been present, that is, the chromatin of which was coarsely reticular or nodular or that contained several small (less than 1μ in diameter) but deeply stained chromatin bodies, or more than one large chromatin aggregate or that were vacuolated. Nuclei which were damaged or overlapped by others, or were tilted or folded so that the position of a chromatin body could not be determined accurately, were also excluded.

Fairly conspicuous chromatin bodies were seen in some of the nuclei satisfying the above criteria; they stained with varying intensity and measured about 1/6–2/3 of the width of the nuclear sector in which they lay. They were usually solitary but nuclei were, for the purposes of this work, accepted as having such a body if they contained not more than one other appreciably smaller chromatin aggregate.

These bodies were either applied to the outer or inner aspect of the nuclear ring or lay free within the nucleus. In all positions their shape could be irregular, but usually they were triangular, trapezoidal or oblong if applied to the nuclear membrane and round or oval if lying free (Fig. 1). Peripherally situated bodies were sometimes bifid[12]. Bodies in other positions were often closely associated with a nucleolus.

Marrows were taken from three males and three females of each of the strains listed above, and all specimens were coded so that the sex of the animals was unknown until the results had been collected.



a *b*

Fig. 1. Metamyelocytes from marrow of a female *C57* black mouse. *a*, cell with no large chromatin body; *b*, cell with a large, peripheral chromatin body

Table 1. Per cent Incidence and Position of Chromatin Body in Rat and Mouse Metamyelocytes

Animal		Chromatin body			
		Peripheral	Central	Free	Absent
Male					
A2	1	2	4	24	70
	2	0	10	28	62
	3	0	6	22	72
C57	1	0	22	10	68
Black	2	0	22	20	58
	3	2	28	14	56
AK	1	2	20	30	48
	2	0	22	20	58
	3	2	6	12	80
WAG	1	0	2	26	72
	2	0	2	14	84
	3	0	2	32	66
PVG	1	0	0	28	72
	2	2	4	36	58
	3	0	0	30	70
Female					
A2G	1	52	12	18	18
	2	42	6	28	24
	3	30	22	28	20
C57	1	34	8	28	30
Black	2	34	24	10	32
	3	32	12	12	44
AK	1	52	8	18	22
	2	38	14	6	42
	3	34	10	16	40
WAG	1	40	2	38	20
	2	40	2	38	20
	3	48	4	34	14
PVG	1	34	10	28	28
	2	34	12	38	16
	3	44	6	16	34

Fifty nuclei were counted in each marrow and the presence or absence of a chromatin body and its position were recorded. Simple inspection of the results showed that marrows could be grouped into two non-overlapping classes: those in which the peripherally situated chromatin body occurred in 30–52 per cent of nuclei and those in which its incidence did not exceed 2 per cent. It was found that all the marrows in the former class were from females and all the latter from males. The results are summarized in Table 1. There was no clear sex difference in the incidence of chromatin bodies in other positions.

It can be concluded that the sex of mouse and rat metamyelocytes may be determined by the incidence of peripherally situated chromatin bodies, a finding that may be of use in studying the fate of inter-specific, inter-strain and intra-strain transfers of marrow.

The photographs were taken by Mr. D. F. Boxall.

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April 13.

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Cytology of *Polygala*

Comparatively little is known of the cytology of the genus *Polygala*. Table 1 gives chromosome counts made on ten European and one South African species, together with previous counts for comparison.

The chromosomes of the *vulgaris–amara* group are very small but not uniform in size (about 0·5–1μ in somatic plates). Those of *P. monspeliaca* are yet smaller but seem more uniform, while those of *P. myrtifolia* are a little larger and not uniform. The chromosomes of section Chamaebuxus range from about ½ to 4μ in length. The resting nuclei of the *vulgaris–amara* group are chromocentric, while those of Chamaebuxus have an abundant reticulum with some chromocentres[7].

In the *vulgaris–amara* group, apart from one rather larger bivalent in *P. calcarea*, individual chromosomes within the complement cannot usually

Table 1. Chromosome Numbers of *Polygala*

	No. of localities	British	Swiss	Other	Previous counts
Section X, Orthopolygala Sub-section 14 (Chodat (ref. 1)), the *vulgaris – amara* group					
P. alpestris Rchb.	6	—	$n = 17$ $2n = c. 34$	—	—
P. alpina Perr. and Song.	1	—	$2n = c. 34$	—	—
P. amara L.	—	—	—	—	$n = 14$ (ref. 2)
spp. *amarella* Cr.	9	$n = 17$ $2n = 34$	$n = 17$ $2n = c. 34$	—	—
P. calcarea Shultz.	6	$n = 17$	$n = c. 17$	$2n = 34$	—
P. comosa Schkr.	9	—	$n = 17$ $2n = 34$	—	$2n = 28$ (ref. 3) $2n = 28–32$ (ref. 4)
P. serpyllifolia Hose.	6	$n = c. 17$ $2n = 34$	$n = 17$	$n = c. 34$	—
P. vulgaris L.	23	$n = 34$ $2n = 68$	$n = c. 34$ $2n = c. 68$	$n = c. 34$ $2n = c. 68$	$2n = 28$ (ref. 4) $2n = 32$ (ref. 4) $n = 24–28$ (ref. 5) $2n = c. 56$ (ref. 4) $2n = c. 70$ (ref. 6)
Section X. Orthopolygala, other sub-sections					
P. monspeliaca L.	2	—	—	$n = c. 19$ $2n = c. 38$	—
P. myrtifolia L.	1	—	—	$2n = c. 38$	—
Section IX. Chamaebuxus					
P. chamaebuxus L.	6	—	$2n = c. 46$	—	$n = 23$ (ref. 7) $2n = 38$ (ref. 4)
P. rayredae Costa	1	—	—	$2n = 28$	—

'*P. oxyptera*' is included under *P. vulgaris*, and does not differ from it cytologically. The British '*P. austriaca*' (Kent) and '*P. amara*' (Yorkshire) are included under *P. amara* spp. *amarella*.

'Other' localities are as follows: *P. calcarea*, St. Andrews Botanic Garden (thought not British material); *P. serpyllifolia* (tetraploid), Corsica; *P. vulgaris* and *P. monspeliaca*, Portugal; *P. myrtifolia*, Antibes Botanic Garden; *P. rayredae*, horticultural, ex. Lausanne.

be picked out. Some of the larger chromosomes in Chamaebuxus can be recognized, however[7]. The largest two in the haploid set of *P. vayredae* have subterminal centromeres and resemble two in *P. chamaebuxus*, and a third nearly as large in *P. vayredae* has a median centromere and again could correspond to a chromosome in *P. chamaebuxus*, but two long chromosomes with terminal centromeres in *P. chamaebuxus* are not present in *P. vayredae* so that while *P. vayredae* may be a parent of *P. chamaebuxus*, a second parent must have contributed these latter chromosomes.

While previous work has suggested a less-orderly situation, the present work indicates that most species of the *vulgaris–amara* group are diploid with $n = 17$, *P. serpyllifolia* has both a diploid and a tetraploid race, and *P. vulgaris* is tetraploid with $n = 34$. Two less-closely related species of section X, *P. monspeliaca* and *P. myrtifolia* (a South African shrub), appear to have $n = 19$, which corresponds with Hagerup's[8] count on *P. erioptera*. In section IX (Chamaebuxus) two seemingly unrelated numbers have been found ($n = 14$ and 23).

Previous counts, except that by the Löves[6] ($2n = c.$ 70 for *P. vulgaris*), are all lower than those reported here. This is undoubtedly largely due to the extreme difficulty in counting the chromosomes of the *vulgaris–amara* group. Counts regarded as individually conclusive were obtained from only a small proportion of the localities examined in this work, though conclusive counts were obtained on each species other than *P. alpina* and clear cases of deviations from the numbers shown were extremely rare.

Baksay's[2] count on *P. amara* refers to a taxon not studied by me, but as her count was made on diakinesis, which I have found deceptive in that some chromosomes are not stainable until the very latest stages, the number could have been higher than she records. If some of the satellites recorded by Larsen[3] in *P. comosa* were independent small chromosomes his plant could be a $2n = 34$ form. Wulff[5], in recording $n = 24$ to 28 for *P. vulgaris*, spoke of difficulties in counting due to meiotic irregularities. Mattick's[4] finding of roughly diploid chromosome numbers in some of her *P. vulgaris* is interesting, but could be due to confusion with the closely related and very variable *P. comosa*. However, more work on European *P. vulgaris* is clearly needed.

Meiotic irregularities have been reported by Hagerup[8] and Wulff[5] in *P. vulgaris* from Denmark and Schleswig Holstein. In the present work they were found to be common in *P. comosa*, but rare in other species. The usual irregularity is the presence of univalents, leading to irregular tetrads. In *P. comosa* the degree of irregularity of the tetrads varied from bud to bud in the same spike (from apparent normality to about 40 per cent of the tetrads abnormal), suggesting that irregularities occurred in response to less favourable weather conditions. Most irregularities in other species could also be attributed to abnormal conditions. It is possible that at times the chiasma frequency may be so far reduced that some potential bivalents are not joined (cf. Elliott[9]).

Variation in the number of chromosomes in pollen grains of *P. chamaebuxus* has been established[7]; but it is not known if this reflects variation in the somatic chromosome number or is an immediate result of irregularities of meiosis. No irregularities in the meiotic process have yet been observed in this species, and more work is required.

A full account of this work, including observations on some interspecific hybrids, is in preparation.

Acknowledgment is made to the Department of Scientific and Industrial Research and to the Conseil d'Etat de Neuchâtel for financial support, and to Profs. D. H. Valentine of Durham and C. Favarger of Neuchâtel for their help and encouragement.

D. R. Glendinning

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Tafo, Ghana.

[1] Chodat, R., "Monographia Polygalacearum" (1891).
[2] Baksay, L., *Ann. Hist.-Nat. Mus. Nat. Hung.*, **7**, 321 (1956).
[3] Larsen, K., *Bot. Notis.*, **109**, 293 (1956).
[4] Mattick, in Tischler, G., "Chromosomenzahlen der Gefasspflanzen Mitteleuropas" (1950).
[5] Wulff, H. D., *Ber. deutsch. bot. Ges.*, **56**, 247 (1938).
[6] Löve, A., and Löve, R., *Arch. Botan.*, **3**, *A*, 381 (1944).
[7] Glendinning, D. R., *Bull. Soc. Neuchâtel Sci. Nat.*, **72**, 161 (1955).
[8] Hagerup, O., *Hereditas*, **16**, 19 (1932).
[9] Elliott, C. G., *Heredity*, **9**, 385 (1955).

PSYCHOLOGY

The Relativity of 'Meaning'

Dr. W. Ross Ashby's communication on this subject[1] is very interesting and may prove to have much technical value, provided that it is possible to achieve a closed system of the outputs with which the brain may respond to the incoming message. This is the crux of the whole question. It is assumed that useful closed formalisms for this purpose are possible. In point of fact, every formalism that we know is made to work by unformalized human adjustments. Without these, the formalism would become so complex that it would lose its value as a shorthand. It would be uneconomical in practice.

It is necessary, then, in setting up a particular formalism to accept the limitation imposed by the practical use of the system and the resources available to use it. Ultimately, this means computing fast enough at an acceptable cost. I have no doubt that there will be invented successful schemes with limited applicability to handle the problem of meaning in translation and other processes that connect messages with action, but no useful purpose will be served by claiming to formalize all the possibilities of human behaviour.

It is very interesting to see recognized in a technical communication that application of a message shows its meaning in the context of the particular occasion, seeing that it is now some time since Wittgenstein first emphasized this thought, and the essential informality of behaviour in responding to ordinary language.

W. H. Watson

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[1] *Nature*, **187**, 532 (1960).

Dr. W. Ross Ashby[1] has made the interesting assertion that the meaning of a message carrying information depends on the context of the message. Thus, if someone sends *A* of two possible telegrams, *A*, "How we wish you were here" and *B*, "The weather is fine", the meaning of the message is different from that of the same message chosen from three messages *A*, *B* and a third one, *C*, "Do come and join us". This may well be true in a coding

system as usable in mechanical information transfer, but it is not true of ordinary human assertions, messages and speech, except possibly in a few odd cases. If a radio message is sent, "The flowers are blooming", and it is the expected message instructing a submarine commander to release a torpedo, it is arbitrary that this particular sentence was prearranged as the message. It could have been a bell sounding, or a man blowing his nose that was the message. If I wish to convey the information that the cat is on the mat, in English, it is not arbitrary what message I use. As the corollary of this, it would be absurd to say that one of the possible meanings of the English sentence, "The flowers are blooming", is that it is an order to fire torpedos. It is similarly absurd to say, as Ashby does, that one of the possible meanings of "How we wish you were here" in English is "We wish to be cordial but do not require your company". The meaning of a simple assertion does not change when the speaker is a liar.

What does change according to the context is what it is possible to infer from the message, or in other words, its information content. Since, however, ordinary English meanings are determined by customary usage, and this particular usage is not at all necessary in constructing a means of communication, it is perfectly feasible, and simple even, to arrange a cipher where the significance of a symbol is altered according to its context among other symbols in the same cipher, as the figure 2 is of different significance in the figures 112 and 211. Thus, for constructing machines or other systems for conveying information by messages, it is possible, but not necessary, that the meaning of a message should depend on its context. It is a mistake to say that this holds for ordinary language.

J. Williamson

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Birkbeck College,
London, W.C.1.

[1] Ashby, W. R., *Nature*, **187**, 532 (1960).

Mr. Williamson points out that in most ordinary language the meaning of a sentence depends on its context only to a trifling or zero degree. Nevertheless, the recognition that the dependence is latent may be worth making. We all know how much Shannon and Wiener contributed by their recognition that the *quantity* of information in a message is a function both of it and of the unsent messages. They showed that a coherent theory can be developed only when the set of messages is regarded as a whole. It may be that a theory of meaning similarly must be based on consideration of the whole set. In such a theory, the sentence the meaning of which depends on context to only zero degree would be regarded as a special (though common) case, degenerate in the way that 'still air' corresponds to the wind that blows at 0 miles per hour.

W. Ross Ashby

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Dr. Ross Ashby[1] is right (defining "right" as "what I think the best people think" and "best" as "tha class of people who think as I do") in his conclusions that no message has intrinsic meaning, and the meaning defined in terms of the set of messages from which it was taken (the sender's meaning) is relatively unimportant. In most cases the message is not consciously composed by an intelligent sender; in many cases our messages arise from non-living matter. The important meaning is the recipient-meaning and this meaning is very personal to the individual recipient (be it man, dog or computer), will vary with instant of reception and will generally decay after reception. It may be defined as the multiplex of correlates stimulated in the recipient by the input message. Even the most intelligent recipient will "know" only in part what this multiplex is. Much of it will not be accessible to his awareness, let alone his analytical senses. Even if he did "know" it, he could not convey it with any absolute certainty to a third person, for the establishment of a common language is a statistical process with a finite residual error-probability even for the most elementary conceptions; and this indeterminacy of meaning applies equally to communications between intelligent beings and to the raw information gathered by the absorption of electromagnetic or acoustic radiation or of vapours from, or contact with, the recipient's environment.

From this we see that no "fact" can ever be "true" in any absolute sense, and the definitions in my first sentence are not flippancies. Nor can any symbolic logic be designed to be absolutely true. Any attempt to reduce "facts" to "atomic" constituents, absolutely transferable, is doomed to failure from the outset, whether the atoms are simple conceptions (which never are simple), or the smallest perceptible increment of intensity (of light or sound) in a particular resolution-cell (of colour, position on the retina, sound-pitch, etc.). For the cross-correlation of such in two recipients will never be more than asymptotic to completeness. Our ability to affect, by intercommunication, each others' actions, and store of correlation patterns determining future actions, will always be on a probabilistic basis. The message transcribed into the memory will sometimes be quite different from the sender's intention, and his-truth will generally differ from my-truth and your-truth.

When we see clearly that there are no intrinsic meanings in words, then we are able to discount such words as 'mind', 'spirit', 'soul', 'psyche', 'freewill', as simply widely used poetic descriptions of some aspect of brain-function and to explain such words as 'truth', 'goodness', 'morality' and 'beauty' in scientific terms, and the conflict with Christian belief is resolved[2]. When we realize, with relief, that there is no intrinsic meaning in the whole, or any part, of the universe, we also see that the beauty of a universe governed by simple laws is no argument in favour of a creator. For the very existence of life (defined as a class of agglomerates of matter so formed as to take advantage, by prediction from stored information, of its environment to increase the probability of perpetuation of its own kind) is utterly dependent on the predictability of that environment. Were the universe not well ordered we could not be here; we can know no universes but beautiful ones, and as things we know not, do not exist, then all universes are inevitably beautiful.

G. F. Clarke

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Royal Aircraft Establishment,
Farnborough,
Hants.

[1] Ross Ashby, W., *Nature*, **187**, 532 (1960).
[2] Lack, David, *Nature*, **187**, 98 (1960).

FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, November 14

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS AND COMMUNICATION SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Tunnel-Diode Applications and Circuitry", opened by Dr. G. B. B. Chaplin and Dr. R. W. A. Scarr.

ROYAL GEOGRAPHICAL SOCIETY (joint meeting with the ANGLO-SWISS SOCIETY, at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Mr. Max Eiselin: "The Ascent of Dhaulagiri".

Tuesday, November 15

INSTITUTE OF PHYSICS AND THE PHYSICAL SOCIETY, ELECTRONICS AND LOW TEMPERATURE GROUPS (at 47 Belgrave Square, London, S.W.1), at 10 a.m. and 2.15 p.m.—Meeting on "Electronic Devices at Helium Temperatures".

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. K. A. Kernack: "Fossils from Fissures".*

UNIVERSITY OF LONDON (at Birkbeck College, Malet Street, London, W.C.1), at 5 p.m.—Prof. J. Wyart (University of Paris): "Diffusion of Atoms (Na, K, Al, Si, O) in Felspars".* (First of two Special University Lectures in Crystallography. Further lecture on November 16.)

INSTITUTION OF ELECTRICAL ENGINEERS, MEASUREMENT AND CONTROL SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. T. Glucharoff: "Discrete Analogue-Computer Compensation of Sampled-Data Control Systems".

UNIVERSITY COLLEGE (in the Anatomy Theatre, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. E. E. Evans: "Environment and Society in the Atlantic Fringe".* (Further lectures on November 17 and 18.)

UNIVERSITY COLLEGE (in the Physiology Theatre, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. J. H. Gaddum, F.R.S.: "The Pharmacological Analysis of Tissue Extracts".*

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. K. S. Kirby: "Nucleic Acids and Cancer".* (Eighth of the fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

ROYAL INSTITUTE OF CHEMISTRY (at Shell Mex House, Strand, London, W.C.2), at 6.30 p.m.—Annual General Meeting.

Wednesday, November 16

INSTITUTE OF METAL FINISHING (in the Recital Room of the Royal Festival Hall, London, S.E.1), at 9.30 a.m.—Symposium on "Nickel-Chromium Plating".

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 2 p.m.—Dr. A. L. Greenbaum: "Fat Metabolism".*

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Scientific Papers.

UNIVERSITY OF LONDON (at Birkbeck College, Malet Street, London, W.C.1), at 5 p.m.—Prof. J. Wyart: "Hydrothermal Synthesis and the Problem of Granitization".*

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.15 p.m.—Prof. M. G. Kendall: "The Natural Law in the Social Sciences" (Inaugural Address).

INSTITUTION OF ELECTRICAL ENGINEERS, SUPPLY SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. E. H. Cox and Mr. H. E. Martin: "Radiocommunication in the Power Industry".

UNIVERSITY OF LONDON (in the Chemistry Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. R. J. Forbes (University of Amsterdam): "The History of the Petroleum Industry: Processing and Products during the Past Fifty Centuries".* (First of three Special University Lectures in the History and Philosophy of Science. Further lectures on November 17 and 18.)

BRITISH INSTITUTION OF RADIO ENGINEERS, COMPUTER GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6.30 p.m.—Lt.-Col. I. W. Peck: "Digital Computing Elements for Instructional Use".

Thursday, November 17

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Special General Meeting, followed by Dr. G. Herzberg, F.R.S.: "The Spectra and Structures of Free Methyl and Free Methylene" (The Bakerian Lecture).

INSTITUTION OF MINING AND METALLURGY (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. Kun Li, Mr. R. W. Livingston and Mr. L. K. Lemke: "Flotation Conditioning of Iron Ore with Petroleum Sulphonate"; Mr. G. J. Brittingham: "Rehabilitation of Chang Hang Copper Smelter, South Korea".

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Annual General Meeting. Prof. G. Kreisel: "Recent Work on the Foundations of Analysis".

UNIVERSITY OF LONDON (in the Physiology Theatre, University College, Gower Street, London, W.C.1), at 5 p.m.—Prof. R. A. Morton: "Ubiquinones and Related Substances—Nature and Biological Functions".* (Further lecture on November 24.)

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. J. C. Kendrew: "The Structure of Proteins".* (Ninth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

SOCIETY OF CHEMICAL INDUSTRY, ROAD AND BUILDING MATERIALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Dr. L. J. Wood: "The Constitution and Properties of Tars and Tar-Polymer Mixtures".

Friday, November 18

BIOCHEMICAL SOCIETY (at the Middlesex Hospital Medical School, Mortimer Street, London, W.1), at 10 a.m.—Colloquium on "Histochemistry". Afternoon session—Original communications on histochemistry and other subjects.

INSTITUTE OF NAVIGATION (joint meeting with the BRITISH INTERPLANETARY SOCIETY, at the Royal Geographical Society, 1 Kensington Gore, London, S.W.7), at 10 a.m.—Symposium on "Navigation for the Early Exploration of the Moon".

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. M. Westergaard (Copenhagen): "Some Fundamental Concepts and Problems in Molecular Genetics".* (Further lectures on November 22 and 23.)

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Prof. J. F. Danielli: "Physicochemical Aspects of Chemotherapy".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Mr. D. E. Broadbent: "The Perception of Speech".

Saturday, November 19

BRITISH INTERPLANETARY SOCIETY (in the Tudor Room, Caxton Hall, London, S.W.1), at 6 p.m.—Mr. D. Andrews: "British Participation in Space Research".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER (with a degree and/or corporate membership of a professional institution, and preferably the ability to offer electrical measurements as a specialist subject) IN ELECTRICAL ENGINEERING at Brighton Technical College, to teach to final degree and diploma standard—The Director of Education, 54 Old Steine, Brighton, Sussex (November 19).

LECTURER (with a good honours degree in pharmacology or physiology, or a qualification in medicine, and a postgraduate qualification in pharmacology) IN THE DEPARTMENT OF PHARMACOLOGY—The Registrar, King's College (University of London), Strand, London, W.C.2 (November 22).

SENIOR LECTURER (preferably with a medical qualification) IN THE DEPARTMENT OF EXPERIMENTAL PATHOLOGY AND CANCER RESEARCH—The Registrar, The University, Leeds (November 22).

LECTURER (specially interested and qualified in the fields of petrology and mineralogy) IN GEOLOGY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, November 25).

LECTURER or ASSISTANT LECTURER IN APPLIED MATHEMATICS—The Secretary, The University, Northcote House, Queen's Drive, Exeter (November 26).

ASSISTANT LECTURER (preferably qualified in physical chemistry) IN CHEMISTRY—The Registrar, The University, Leicester (November 30).

ENTOMOLOGIST, Scientific Officer or Senior Scientific Officer grade (with a good honours degree), to study the effect of insecticides on insect populations—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (November 30).

LECTURER (with a good honours degree or equivalent and preferably teaching experience) IN THEORETICAL PHYSICS; and a LECTURER (with a good honours degree or equivalent and preferably teaching experience) IN PHYSICS at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, November 30).

READER IN ANALYTICAL AND INORGANIC CHEMISTRY; and a LECTURER or ASSISTANT LECTURER IN INORGANIC CHEMISTRY—The Clerk to the Governors, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (November 30).

SCIENTIFIC OFFICER (with a first- or second-class honours degree in mathematics with statistics, or a diploma in statistics) IN THE STATISTICS SECTION, for duties mainly concerned with the design and analysis of field experiments—The Secretary, The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen (November 30).

BIOLOGIST (with Ph.D., M.Sc., or B.Sc. in wildlife management or zoology, with experience in independent water-fowl research) IN THE WILDLIFE BRANCH, Department of Internal Affairs, Wellington, New Zealand, to carry out life-history studies of mallard and native grey duck populations or such other projects as may be assigned—The High Commissioner for New Zealand, 415 Strand, London, W.C.2 (December 1).

PROFESSOR OF AGRICULTURE AND DIRECTOR OF THE COLLEGE FARM—The Secretary and Registrar, University College of North Wales, Bangor, North Wales (December 1).

BEYER CHAIR OF APPLIED MATHEMATICS—The Registrar, The University, Manchester 13 (December 3).

LECTURER IN THE DEPARTMENT OF CLASSICAL ARCHÆOLOGY—The Secretary, The University, Edinburgh (December 5).

HEAD OF THE DEPARTMENT OF VIROLOGY, for duties which will include responsibility for the development of the Department for the study of animal viruses in close collaboration with the Departments of Pathology, Bacteriology and Biochemistry—The Secretary, Field Station, Agricultural Research Council, Compton, near Newbury, Berkshire (December 15).

LECTURER or ASSISTANT LECTURER IN PHYSICS at the University of Malaya, Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (December 15).

LECTURER (specialized, or prepared to specialize in the pre-history of South-East Asia and/or Oceania, and able to teach elementary physical anthropology) IN ANTHROPOLOGY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, December 15).

LECTURER (with at least a first-class honours degree in chemical engineering) IN CHEMICAL ENGINEERING at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, December 19).

SENIOR LECTURER IN BOTANY and a LECTURER IN BOTANY at the University of Adelaide, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, December 20).

SENIOR TUTORS (2) IN PURE MATHEMATICS at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, December 20).

SENIOR RESEARCH FELLOW or a RESEARCH FELLOW IN THE DEPARTMENT OF NUCLEAR PHYSICS, Research School of Physical Sciences, Australian National University, to work either in the field of nuclear spectroscopy or on photonuclear problems—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, December 30).

CHAIR OF ZOOLOGY in the University of Malaya, Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (December 31).

TURNER AND NEWALL FELLOW (preferably under 28 years of age) at Newcastle or Durham, for research in Engineering, Inorganic Chemistry or Physics, or an allied science—The Registrar, University Office, 46 North Bailey, Durham (January 31).

IMPERIAL CHEMICAL INDUSTRIES LIMITED RESEARCH FELLOWS IN BACTERIOLOGY, BIOCHEMISTRY, BIOMOLECULAR STRUCTURE, BOTANY (PLANT BIOCHEMISTRY AND BIOPHYSICS), CHEMICAL ENGINEERING, CHEMISTRY, CHEMISTRY OF LEATHER MANUFACTURE, CHEMOTHERAPY, COLOUR CHEMISTRY AND DYEING, ENGINEERING (CIVIL, ELECTRICAL AND MECHANICAL), FUEL AND REFRACTORIES, GEOLOGY (including GEOCHEMISTRY), METALLURGY, MINING (SELECTIVE FLOTATION AND GEOPHYSICAL SURVEYING), PHARMACOLOGY, PHYSICS, PHYSIOLOGY OR TEXTILE INDUSTRIES (CHEMISTRY OF HIGH POLYMERS (KERATIN AND MAN-MADE FIBRES), TEXTILE TECHNOLOGY AND TEXTILE ENGINEERING)—The Registrar, The University, Leeds 2 (February 17).

ANALYTICAL CHEMIST (with an honours degree in chemistry or Associate Member of the Royal Institute of Chemistry, and preferably postgraduate experience in a public analyst's laboratory or veterinary research establishment), at the Veterinary Research Laboratories, near Nairobi, Kenya, to analyse animal food-stuffs for legislation purposes and carry out research in nutrition—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.67/7/017.

ASSISTANT LECTURER IN PHYSIOLOGY—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8.

ASSISTANT VIROLOGIST (with previous experience in virological techniques) to undertake research on rubella and molluscum contagiosum, with particular reference to the isolation of their causal agents—Head of the Virology Department, The Lister Institute, London, S.W.1.

PRINCIPAL LECTURER (qualified to teach to final degree or diploma in technology standard) IN THE DEPARTMENT OF BIOLOGICAL SCIENCES—The Registrar, Bradford Institute of Technology, Bradford 7.

RESEARCH ASSISTANT (with a science degree in physiology, biochemistry or a cognate subject, and preferably experience in pharmacological or biochemical techniques) TO THE PROFESSOR OF PHARMACOLOGY, for work related to the assay of chemical transmitters in tissues and body fluids—Prof. G. Brownlee, Department of Pharmacology, King's College (University of London), Strand, London, W.C.2.

SUPERINTENDENT OF CHILDREN'S ZOOS in the L.C.C. Parks Department (preferably zoologist with some years practical experience)—Chief Officer, Parks Department (A.1/N/2736/11), County Hall, London, S.E.1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Factors in Mental Evolution. By The Rt. Hon. Lord Adrian. (The Romanes Lecture delivered in the Sheldonian Theatre, 24 May 1960.) Pp. 19. (Oxford: Clarendon Press; London: Oxford University Press, 1960.) 3*s*. 6*d*. net. [48

British Archaeology: a Book list. Pp. 43. (London: Council for British Archaeology, 10 Bolton Gardens, S.W.5, 1960.) 5*s*. 6*d*. [48

Société Jersiaise. La Cotte-de-Saint-Brelade, Jersey, British Channel Islands. Excavation of a Pre-Mousterian Horizon, 1959–1958. By Father Christian Burdo, S. J., Ph.D. Pp. 77+8 plates. (Jersey, Channel Islands: Société Jersiaise, The Museum, 9 Pier Road.) 7*s*. [48

Ministry of Housing and Local Government. Smoke Control (England and Wales): Summary of Programme submitted by Local Authorities for the Establishment of Smoke Control Areas. Pp. 19. (Cmnd. 1113.) (London: H.M. Stationery Office, 1960.) 3*s*. net. [48

General Register Office. Studies on Medical and Population Subjects. No. 14: Morbidity Statistics from General Practice, Vol. 2: (Occupation). By Dr. W. P. D. Logan. Pp. vii+269. (London: H.M. Stationery Office, 1960.) 25*s*. net. [48

Forestry Commission. Booklet No. 5: Conversion Tables for Forest Research Workers. Pp. viii+64. 4*s*. net. Forestry Commission Guide to Westonbirt Aboretum. Pp. 8. 6*d*. net. Forest Record No. 43: Establishment Methods for Poplars. By J. Jobling. Pp. 16. 2*s*. net. (London: H.M. Stationery Office, 1960.) [88

Department of Scientific and Industrial Research. Building Research 1959: The Report of the Building Research Board with the Report of the Director of Building Research. Pp. iv+81+16 plates. (London: H.M. Stationery Office, 1960.) 7*s*. net. [88

Other Countries

Report of the South African Museum for the year ended 31st March 1959. Pp. ii+19+5 plates. (Cape Town: South African Museum, 1960.) [38

United States Department of Commerce: National Bureau of Standards. Monograph No. 10: The "1958 He^4 Scale of Temperatures". By F. C. Brickwedde, H. van Dijk, M. Durieux, J. R. Clement, and J. K. Logan. Pp. ii+17. (Reprinted from the *Journal of Research of the National Bureau of Standards—A. Physics and Chemistry*, Vol. 64A, No. 1, January–February, 1960.) (Washington, D.C.: Government Printing Office, 1960.) [38

East Africa High Commission. East African Agriculture and Forestry Research Organization. Record of Research for the period 1st January to 31st December, 1959. Pp. vii+91. (Nairobi: Government Printer, 1960.) 8*h*. 3/50. [38

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